

nature

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Behind the scenes at NAS

IN May 1966, the Atomic Energy Commission received some sharp criticism from a committee of the National Academy of Sciences concerning its plans for getting rid of radioactive wastes. It wasn't the first time that the AEC had been stung by that particular committee and it was none too pleased. It promptly suppressed the report, informed the Academy that its advice on waste disposal was no longer needed, and said that its contract with the committee would be terminated the following year.

What did the Academy's leadership do to prevent the committee's expert advice on a matter of public importance from being ignored by an agency which didn't want to listen? It tacitly acquiesced in the suppression of the report by doing little to bring it to public attention, then it entered into negotiations with the AEC, as a result of which the offending committee was disbanded and the Academy appointed in its place a committee consisting almost entirely of former AEC officials and consultants. The AEC then renewed its contract with the more sympathetic panel.

That sordid episode is one of a number of skeletons in the cupboard of the National Academy of Sciences which are laid bare in a book published this week (*The Brain Bank of America*, by Philip M. Boffey, McGraw-Hill, \$10.95). The fruit of an investigation begun in 1971 and supported by Ralph Nader's Center for the Study of Responsive Law, the book represents a remarkable blend of investigative reporting and careful scholarly analysis. It also shatters a few popular myths about the scrupulous objectivity of scientists called on for advice on matters of public policy.

Like many other learned societies, the Academy is a very prestigious body. Its members are elected for their outstanding contributions to scientific re-

search and represent the élite in American science. Indeed, for the few dozen scientists who are accepted into the Academy each year, the honour is generally second only to being awarded the Nobel Prize.

But, unlike its counterparts in many other countries, the Academy is much more than just a scientific hall of fame. It attracted Nader's tender scrutiny because it provides massive amounts of advice, under contract chiefly to the federal government, on issues which touch the lives of virtually every American (and, indirectly, the lives of many people outside the United States as well). It operates literally hundreds of advisory committees concerned with topics as diverse as the safety of food additives, the need for new military hardware, the effects of SSTs on the ozone layer and the nutritional quality of dog food.

Established by an Act of Congress signed by President Abraham Lincoln in 1863, the Academy's government advisory work is performed through a body called the National Research Council (NRC), which is essentially the Academy's operating bureaucracy. (Two other organisations—the National Academy of Engineering and the Institute of Medicine—are also officially part of the Academy and operate through the NRC.) Briefly, when the Academy accepts a contract to undertake a study, the National Research Council reaches out into the scientific community and pulls together a committee of experts (who are not necessarily Academy members) which eventually turns in a report. After review by a committee of Academy members, the report is passed on to the agency which asked for it.

Reports which bear the Academy's name tend to be influential documents because although they may be written by scientists who are not members of the Academy, they carry some of the

Academy's considerable prestige. Moreover, because the Academy is an independent body with no particular axe of its own to grind, its advice is assumed to be untainted by the workings of vested interests.

But Nader was particularly concerned because the Academy had traditionally operated behind closed doors—a situation in which it is difficult for an outsider to judge how well it lives up to its reputation as an impartial adviser to the federal government.

Philip M. Boffey, a former reporter for the *Wall Street Journal* and *Science*, who conducted the investigation under Nader's sponsorship, has carefully peeled back the layers of secrecy surrounding the Academy's operations, leaving its biases and failings nakedly exposed. Rather than attempt to do a head count of all the skeletons he could find in the Academy's cupboards, Boffey applied his reporter's talents to seeking out the part the Academy plays in a number of important national issues involving science and technology. Certainly, it can be argued that his choices neglect some of the Academy's better works and Boffey himself acknowledges that the Academy has produced some reports of "outstanding quality", but Boffey's diggings have unearthed a number of flaws in the organisation's performances which seem to have broad implications.

Boffey's chief criticism is that the Academy is so dependent on the federal government for operating funds—it has a staff of more than 1,000 to support and the government provides some 80% of its budget—that it is too easy for government agencies to manipulate the Academy's committees. Specifically, Boffey suggests that there is sometimes a tendency for Academy committees to mute their criticisms in order not to lose future government contracts, agencies occasionally seek the Academy's support for decisions which have



already effectively been taken, and the Academy is frequently asked to comment only on specific aspects of a policy rather than to examine the policy itself.

Boffey cites, and carefully documents, a number of examples of this "master-servant relationship" between government agencies and the Academy. The Academy's response to the AEC's threat to cut off funds for the committee on radioactive waste disposal is one such example. Another is the Academy's early role in the SST debate.

Although the Academy played no official part in the crucial decisions leading up to President Kennedy's 1963 announcement that building an SST would be a national goal, it was hastily brought in a year later when the government's sonic boom tests ran into public opposition. Asked to make a rather narrow study of the sonic boom problem, the Academy, according to Boffey, "showed a disquieting tendency to place itself on the FAA's (Federal Aviation Administration's) side of the controversy rather than act as an independent source of advice". An early Academy report even suggested that the government should launch a public relations effort to develop "attitudes sympathetic to the problem" among the general public. And a later report engendered so much controversy that 189 Academy members signed a petition asking for alleged inaccuracies in it to be corrected.

As for the charge that the Academy is sometimes used by government agencies to provide prestigious backing for policies which have already been decided, the Academy's role in the 1969 ban on the artificial sweetener, cyclamate, seems to be a case in point. When reports that cyclamates may be carcinogenic surfaced in October, 1969, the Food and Drug Administration was reluctant to act without the Academy's approval. With some hesitation, an Academy committee agreed to examine the relevant data at a meeting with FDA officials on October 17, and the next day Jesse Steinfeld, the Surgeon General, was able to announce at a press conference that "after a thorough review", the Academy has "independently confirmed our interpretation of the information and recommended that we remove cyclamates from the list of approved substances for general use".

Another important charge which Boffey makes is that the Academy is sometimes subject to corporate influences while it gives short shrift to the concerns of non-establishment scientists such as environmentalists and so-called public interest groups. He notes, for example, that the Academy committee responsible for advising on the safety of foods "has long and very close ties with the food and chemical

industries", even to the extent that the food industry has financed its work, and some committee members were receiving research grants from the food industry while they were passing judgment on the safety of food additives. Boffey also documents the fact that key sections of a report on the hazards of airborne lead were drafted by an employee of a company which produces lead additives for gasoline, and he quotes statements by members of Academy committees advising on pesticides which are highly critical of environmentalists. Though he cites no instances in which committee members benefited directly from the advice they offered, Boffey suggests that he "found, surprisingly, that the Academy's advisory reports often fall short of the very high quality one would expect from the nation's pre-eminent scientific organisation; many, in fact, are mediocre or flawed by bias or subservience to the funding agencies".

But perhaps Boffey's most telling criticism is that, although the Academy is ideally placed to take the lead in debates on important national issues, it seldom does so. It was left to others, for example, to raise questions about the dangers of overuse of pesticides, to lead an investigation into the harmful effects of spraying millions of gallons of herbicides on Vietnam, and to examine such issues as nuclear reactor safety, automobile safety and the improvement of health care.

Boffey cites several reasons for the Academy's lack of leadership on such issues—including the fear that the Academy's reputation might be muddled if it gets involved in controversial political issues, and "reluctance on the part of some scientific leaders to oppose the government too vigorously lest they lose their potential ability to influence decisions." But he suggests that the chief reason is "undoubtedly the Academy's orientation toward serving rich and powerful institutions that do not generally encourage activism in behalf of reform". Although the Academy does get involved in important national issues, it frequently does so only at a late stage, and then only in response to specific requests for advice on narrowly defined topics, and it has "seldom, if ever, issued the kind of seminally significant document which revolutionises a nation's perception of public issues", Boffey contends. Another reason why the Academy rarely produces broad-gauged studies off its own bat is that it lacks free funds to do so.

What does the Academy leadership think of Boffey's criticisms? Academy President Philip Handler politely refused an interview to discuss the book on the grounds that he has had time

only to skim the introduction and conclusion, and he is saving the rest for his summer reading. Nevertheless, in an address to the Academy membership in April, Handler called the book "a careful analysis of our defects" and he added that "the flaws which are detected by and large, it seems to me, are relatively minor, albeit real. And I think that we have erected arrangements such that most of those are unlikely to recur".

Handler has, indeed, instituted a number of reforms which should improve the Academy's operations. The NRC bureaucracy has been reorganised and streamlined, all reports issued in the Academy's name are now reviewed for accuracy by a committee of Academy members, committee members are screened for possible sources of bias, all unclassified Academy reports are now available for public inspection, and a small fund has been established to enable the Academy to launch its own studies on important topics.

But Boffey argues that those reforms are not sufficient because they "do not provide a fundamental solution to the Academy's major weaknesses: its servant-master relationship to the government agencies and industrial interests which provide financial support." He suggests that the Academy "should designate itself a national ombudsman . . . and every Academy committee should approach its task as if it were representing the public rather than offering consulting services to a particular agency". Most important, it should scale down its activities to a level it can support from its own funds, accepting only those tasks which are deemed to be of great importance, demand the highest technical competence and require independence of judgment. It should also discard its belief that candour thrives only in secrecy, and throw open its operations to public scrutiny, and it should actively solicit ideas from activist groups, Boffey suggests.

Finally, Boffey notes in the preface that "if this book leaves one message with its readers, may it be the realisation that expert pronouncements must never be taken on faith. They must be subjected to the same intense scrutiny and questioning as the public applies to political pronouncements. This is no trivial matter, for the problems we highlight in this book are by no means unique to the National Academy of Sciences. They exist in even more virulent form throughout thousands of organisations, advisory groups and institutions that affect our lives".

The myth of the objectivity of scientists on matters of public policy is indeed an enduring one.—COLIN NORMAN.

international news

THE UK's National Radiological Protection Board is setting up a comprehensive register of all UK radiation workers. Although radiation dose records are already kept by individual employers in the radiation industries, these data will, for the first time, be collected together in the register; epidemiological studies of radiation workers will then be much easier to carry out than they have been in the past.

The success of the register will depend on the cooperation of the different sectors of industry and of the UK Office of Population Census and Surveys (OPCS) which will play a vital part in tracing the medical histories and causes of death of those registered. Discussions are still in progress with the OPCS and with employers and trade unions, so final plans are not yet complete, but the NRPB is optimistic about the possibilities. It hopes to be able to make a start within a few months by putting plutonium workers on the register, and then to follow this up by adding all other radiation workers to its files. The register will record internal exposure from ingested radioactive substances as well as external exposure.

A compelling reason for setting up such a register, according to the NRPB, is that it should help to establish the long term effects of low doses

Register for UK radiation workers

from Peter Goodwin

of radiation. Although there is every reason to believe, on the basis of experience in industries throughout the world which are involved with radiation, that the present permissible dose levels for radiation workers are safe, it has been difficult to establish this with certainty in the absence of a comprehensive record. Individual radiation workers move from job to job, and also take jobs in industries not concerned with radiation, so dose records are often incomplete. The permissible dose levels accepted at present are mostly calculated by extrapolation from studies made among such populations as the Japanese exposed to radiation from the Hiroshima and Nagasaki nuclear bombs and those exposed to radiation for medical purposes. But as such populations were subjected to far higher doses than are experienced in occupational situations, there is always some doubt as to whether the extrapolation is reliable.

The register is planned to be both

retrospective and prospective. Retrospective epidemiological studies can be difficult to carry out because employers do not normally keep track of their employees after their employment has ended. Also employees' National Health Service (NHS) Numbers are often lacking on the company records. A further complication arises from the 'notional dose' phenomenon: the practice of some companies of estimating employees' doses in the absence of genuine records. As 'notional doses' are generally high, to give a margin of safety, it is possible that retrospective studies can give the impression that a relatively high occupational dose is less harmful than it actually is. In spite of these difficulties, says the NRPB, the register should greatly facilitate retrospective investigations of possible radiation effects. Prospectively, it will ensure that all future dose records contain all relevant information, such as the employee's NHS number, so that his death certificate can ultimately be located, as well as date of birth, address, and other facts relevant to tracing his medical history. But as possible carcinogenic effects of low dose radiation are expected only to show up in the very long term, the prospective records are unlikely to be useful for assessing the safety of dose levels for many years, perhaps decades. □

THE vulnerability of the research teams attached to the smaller drug companies was made painfully obvious recently to the research staff of Aspro-Nicholas, the UK subsidiary of the Australian pharmaceutical concern. The entire research staff of around 170 scientists and technicians at the Nicholas Research Institute at Slough were told that they had lost their jobs as the company had decided to close down its research effort completely. Although Nicholas does development work and has manufacturing interests in several countries, all basic new product research has been concentrated in the UK and will now cease. A small unit in Australia which carries out long-term toxicology studies will still remain in being.

Although possible cuts in the research budget and even a few redundancies had been envisaged by the staff as a result of the prevailing economic conditions, the whole-

sale closure of the laboratories came as a complete shock to those involved. The reasons given by the company were the rising costs of research and the decreasing chances of a significant breakthrough. About 120 of the staff have been given one month's notice and others have been invited to stay on for a few months to wind up projects.

The view that investment in pharmaceutical research is now yielding a steadily diminishing return is to some extent supported by surveys from the United States. In 1959, 63 new drugs were put on the market there; in the early 1970s, in spite of a trebling in research funds, an average of only 15 new drugs each year appeared between 1971 and 1973.

This is small comfort to the staff of Nicholas, however, who felt that they had several promising lines of research which could have started to show a return during the next few years. The research effort at

Nicholas has only been going at a significant level for about six years, a relatively short time in applied pharmaceutical research. Research was in progress into analgesics, anti-inflammatory agents, drugs affecting the central nervous system such as anti-depressants and minor tranquillisers, and cardiovascular drugs. Several compounds had reached the drug candidate stage. Nicholas was also evaluating several compounds initially developed in university laboratories.

A spokesman for the Association of the British Pharmaceutical Industry said that the association had been issuing warnings for some time that the high cost of research and the increase in time needed to bring a new product on to the market, together with a decline in profitability of the industry generally, has created a situation in which the research and development needed to produce new medicines is being placed in jeopardy.

THE belief that Canada is rich in natural resources is generally accepted by Canadians as well as others, and is certainly widely held by their neighbours south of the border. It is, in fact, one basis of the USA's wish for joint control of North American resources.

It is thus interesting to hear from a professor of geology at Carleton University, F. Kenneth North, that this idea is largely illusory. "Canada," Dr North told a recent Conference on Technology and Canadian Foreign Relations at this university in Ottawa, "is the only energy-deficient nation in the world which believes itself to be energy-abundant and behaves accordingly."

Dr North has a habit of making flat, uncompromising statements, and he has been making quite a few of them regarding Canada's energy supplies in recent months. The kind of thing he told the Carleton conference was just what he has been telling federal authorities, although there is not much evidence that they have been listening.

Perhaps this is because they do not want to believe him. His message is that energy supplies—particularly oil—have been vastly overestimated and that, regardless of what action is taken, Canada faces a severe energy crisis before the end of the 1970s.

"No matter how carefully we establish our priorities, no matter how expeditiously that conversion to other energy forms can be brought about, no matter how successful our conservation measures may become, social and economic disruptions on a vast scale are inevitable", he says. "The United States will be a much less affluent and comfortable place than it has been. Canadians should be wondering whether large parts of their country might not have to be evacuated."

That is pretty strong stuff, and the recourse of some who hear it is simply to recoil into their natural optimism. Philip H. Tresize, another delegate to the Carleton conference (organised by the Norman Paterson School of International Affairs), did just that. Now a senior fellow at the Brookings Institute and a former US ambassador to the OECD, Mr Tresize replied: "I am not impressed with the idea we're running out of resources . . . I can't challenge Dr North, but the world is made up of little else than raw materials, and our history has been that we've consistently found new resources, and I have no reason to believe this will not continue to the end of time."

Dr North had more facts than that at his disposal. Canada has always been regarded by her American neighbours as "some kind of resource cornucopia," he said. "But Canada is not remarkably rich in primary resources in proportion to its size. The number of really large mines in Canada can be counted on the

Is Canada an energy-deficient nation?

from David Spurgeon, Ottawa

fingers of one hand, and we have no very large oil or gas fields. There are individual copper mines in Chile and Africa capable of producing more new copper than all Canadian mines put together. Many of the richest gold and silver camps are in the western Cordillera of the Americas.

"Though we have 7% of the Earth's land area, and more than 7% of its area of continental crust, we have less than 2% of its conventional oil," said Dr North. "Even our vast reservoirs of fresh water are easily misrepresented. Far too much of it lies on the surface in easily polluted lakes; for a land of Canada's size, we are short of good subsurface aquifers because so much of our territory is either Precambrian shield or impermeable igneous rock.

"Canada owes her outstanding mineral production to her geographic and economic relationship to the United States. We have allowed them to establish our country as their resource hinterland. Canada's gross mineral production constitutes 7% of our gross national product. Ninety per cent of it is exported. Of all Canadian exports, of all materials to all trading partners, nearly one-sixth is of minerals and fossil fuels to the United States."

Canada's total known remaining oil reserves represent less than 15 months of present North American consumption, Dr North said. The proven tar sands reserves represent for North America an extension of four years in the life of its reserves—not hundreds as many imagine. Canada's total known natural gas reserves, including those in the Arctic, which may never be made available, constitute a supply of about 30 months at present North American rates of consumption. And the reserves of gas so far known in the Mackenzie Delta, after nine years of drilling, would keep the proposed 48-inch pipeline filled for 4½ years.

North's pessimism extends beyond Canada, however. Outside the Communist world, the rate of finding oil has shown a perceptible decline since 1963, he says, and outside the Persian Gulf, the heyday of which is now past, the 'free-world' has found only four basins having recoverable reserves of oil large enough to satisfy a single year's global demand.

The only new basins capable of yielding prolifically are in remote or unreliable regions, chiefly offshore, and statistical probability studies show that the Earth's crust is "very unlikely to possess more than a handful of prolific basins still to be discovered. Even

if each of these is presumed to be as rich as the Alberta basin, or the North Sea, we are looking at a quantity of new oil, from new regions, very much smaller than the quantity we have already produced and consumed."

This leads Dr North to predict that the level of world oil production will start to decline in about 1985, not halfway through the 21st century, as many authorities maintain.

Similarly, he says it is statistically unlikely that North America can possess more than one or two potentially prolific basins that are still undiscovered. Canada's rate of production must start to decline in a year or less, because reserves have already been declining for five years and the rate of addition to reserves has been declining for 10 years.

"Starting next year, our capacity to provide domestic oil will decline at an average rate of 110,000 barrels a day per year. Unless all demand growth can be curbed, therefore, even a new tar-sand plant every year would be incapable of maintaining Canadian self-sufficiency in oil. By 1981, our deficit will reach 1 million barrels a day . . . It is unlikely—almost inconceivable, in fact—that tar-sand production will reach even half that amount by then."

Such doom-saying did not impress Mr Tresize. If we run out of one resource, he said, human experience teaches us that we can have confidence we will find substitutes that will do just as well. □



A hundred years ago

DR FOREL, of Lausanne, has for several years been investigating what are known as the *Seiches* of the Lake of Geneva. *Seiche* is applied locally to certain oscillatory movements which are occasionally seen to occur on the surface of the lake. The phenomenon had been investigated by previous observers, among others by Saussure and Vaucher, who attributed the phenomenon to variations in atmospheric pressure; in this, Forel, who has most minutely investigated the phenomenon, agrees with them. The phenomenon is found to occur on other Swiss lakes, and Forel believes it will be found in all large bodies of water. Indeed, he recognises in the *Seiche* probably the most considerable and the grandest oscillatory movement which can be studied on the surface of the globe.

from *Nature*, 12, 134; June 17, 1875

correspondence

Integrity in science

SIR,—In a leading article in *Nature* (September 29, 1972) there occurs the following passage relating to my then recently published book, *Science at the Crossroads*: "Professor Dingle goes on to complain that a promised leading article rounding off the correspondence has never appeared, apparently oblivious of the way in which his promises to 'bring discredit on the journal' may have discouraged the judicious summing-up for which he asked". Although this was misleading, I ignored it because my primary aim, as my book makes clear, was to ensure that respect for moral integrity should take precedence of all other considerations in scientific practice, and I did not wish to add the relatively trivial question of my own reputation to the already excessive number of possibilities that had been exploited to divert attention from that. On the one occasion on which I did defend myself, the reason was that that had become my only means of establishing beyond doubt that the statements in my book were true. I had then, as now, no object but to make known the actual degree of awareness of moral responsibility within the scientific community, with the ultimate aim of raising it.

I hoped that this feature of the situation had thus been finally disposed of. A recent incident now makes it necessary, however, for me to state the facts relating to the passage quoted above. I have a complete record of my correspondence with *Nature* on this matter, and the only possible basis for what is described as "his [Dingle's] promises to bring discredit on the journal [*Nature*]" is the following extract from my letter of May 20, 1969 to the then editor: "It is now about nine months since I sent you my reply to Syge: it remains unpublished, and it is becoming increasingly difficult to present *Nature's* attitude without reflecting discredit on the journal. I repeat that I do not want to do this, but I must get the truth brought out, whatever it involves, and you are leaving me no alternative." There are many other passages in the correspondence in which I strongly urged the editor not to make necessary revelations which would lower the high reputation which *Nature* had acquired under the editor-

ship of Lockyer and Gregory, which I had a strong desire to preserve, reinforced by my long and intimate association with the journal in Gregory's days. The reference to "my reply to Syge" is explained in my book; here it is necessary only to say that what is described as "the judicious summing-up for which he [Dingle] asked" was not asked for by me, but was spontaneously promised by the editor on November 24, 1969 (six months after my alleged "promises" which, it is stated, "may have discouraged" its appearance) as a substitute for my reply to Syge, which I would far rather have had and which might have settled the whole matter there and then (for details see my book). This, like the "judicious summing-up", has never been published.

This incident has implications for the future as well as, and far more important than, those for the past. Those who share my view of the supremacy of moral obligations over considerations of expediency in science can no longer remain unconvinced that the continued evasion or ignoring of the simple question concerning the rates of two clocks in uniform relative motion which I have asked many times—conspicuously in my book and lastly, so far as *Nature* is concerned, in my letter there of August 31, 1973 (hereinafter called L)—constitutes a violation of the basic ethical principle of science, with the gravest implications. If evidence of this were still needed, it would be given by the existence of a letter, signed by 12 persons highly qualified in the fields of physics, astronomy, space research, electrical engineering, the Open University, psychiatry, archaeology, religion (three separate branches of the Christian Church of world-wide repute), philosophy, law—who constitute a small fraction of the number who have expressed accordant convictions. This letter asks, in view of the objective facts disclosed in my book, for authoritative reassurance, by the provision of a direct answer to my question, that integrity is still preserved within the scientific community. It has not succeeded in being published in any journal where its appearance would be appropriate and effective. The signatories, many of whom claim no technical qualifications in relativity theory, see clearly that the question asked is

perfectly intelligible without technical knowledge, and that the failure to provide an equally intelligible answer is unmistakably a moral failure.

Notwithstanding the obviousness of this to any reader of my book or of L, no comment on L of any kind has appeared in *Nature* until Kilmister's recent offer of a solution to the problem in the issue of December 6, 1974. In one of the *Three Musketeers* stories, General Monk's skill in diplomacy is said to be shown by his practice, when wished "Good morning", of not replying immediately but waiting twelve hours and then saying "Good evening". Kilmister seems to have emulated Monk. In L, in an attempt to prevent repetition of earlier evasions of my question, I repeated that it had nothing to do with, among other things, "coordinate systems or frames of reference". Kilmister waits 15 months and then says (*Nature*, 252, 439; 1974) "The basis of Dingle's long-standing arguments with the relativists seems to be his insistence on the arbitrary nature of inertial frames." L is, however, still available for reference, and anyone may evaluate Kilmister's judgment by looking it up. He will see that there are only two possible ways of answering my question which are compatible with the ethical demands of science: they are (1) the completion by a single phrase of the following sentence: "The slower-working of the two clocks, A and B, mentioned in the question—which, as shown in L and elsewhere, the theory requires actually, and not merely apparently, to work at different rates—is that which . . ."; (2) acknowledgment that the theory permits no means of proving that A works more slowly than B that is not applicable with equal validity to prove that B works more slowly than A, and that, since this involves a physical impossibility, the theory is untenable.

I think it must now be clear to every reader, whether physicist or not, whose mind has not already been finally closed on this subject, that the basic issue here is a moral one, all intellectual, metaphysical and mathematical considerations being secondary, and that the possibility of continued belief in the moral integrity of the scientific community, the social significance of which in the present age needs no pointing out, depends on the early

provision, by some body or person generally understood to be qualified to speak with authority on the theory, of one of these answers, unobscured by additional verbiage, diagrams or arrays of symbols of any kind.

HERBERT DINGLE

Purley, Surrey

SIR,—Your readers should first know the circumstances in which this exchange of correspondence is published. Professor Dingle has complained that the leading article of September 29, 1972, was defamatory of him, but has undertaken not to take legal action if you publish a letter (above) which I consider might be damaging of me, your predecessor, without the following explanation, however tedious.

I know of nobody who wishes to damage Dingle or his reputation. I would be sorry, but at the same time exceedingly surprised, if the leading article to which he refers had been read in that sense. It was a forthright article but pallid compared with some of the polemics with which Dingle has entertained the readers of *Nature* in the past quarter of a century.

On one small point, whose relevance to his complaint is debatable, Dingle is right. His promise to “bring discredit” on *Nature* was made before and not after I rashly volunteered to write a leading article explaining why his argument is false. I abandoned this project after letters from him such as that of April 6, 1971, in which he wrote that “action on your part even now would make a full exposure of the ethical aspect of the matter unnecessary”. It would no doubt have been more courteous to have told him explicitly that, as far as I was concerned, his dealings with *Nature* were at an end, but the correspondence had become offensive and repugnant. Dingle may not appreciate how the manner in which he has pressed his case has often forfeited him the indulgence that one of his age and wit would ordinarily command.

Your readers should know that the ‘recent incident’ referred to in Dingle’s letter was my comment to the editor of an overseas journal that the author of an article submitted for publication and purporting to be an objective account of this business had at no time sought to obtain my side of the story.

Dingle is wrong to claim that this is an ethical issue. His view that special relativity is a house of cards has been widely aired and amply refuted. I note that he does not refer to the simple statement of the reasons why he is mistaken presented in the leading article of which he now complains.

Dingle’s error is primitive, as can be told from his penultimate para-

graph. He says it is a “physical impossibility” that clock A should work more slowly than clock B and that the reciprocal should also be true. Let him measure time by the frequency of a laser, and suppose two identical lasers pointing at each other are in relative motion. The light received at each laser will be out of tune with the local standard and the phenomena observed at the two lasers will be identical. Knowing that the two lasers are identical, each observer can construct an algorithm so as to infer what time is being kept by the other and will rediscover the familiar and the relativistic Doppler correction. In other words, each frequency comparison will show that the distant laser is “running slow”. Dingle’s assertion that this is a physical impossibility is tantamount to the assertion that it is physically impossible for the velocity of light to be independent of its direction.

Dingle’s confusion stems from his assertion that special relativity requires that the differences of rate should “actually and not merely apparently” occur. The truth, of course, is quite the opposite. The theory is cast in that positivist mould in which no meaning can be attached to physical quantities unless they are observed or made “apparent”. It explicitly rejects the use of physical quantities which cannot be measured. By supposing that there can be measures of time more “actual” than those based on measurement, Dingle is simply asserting that he holds to the pre-relativity notion of absolute time.

Dingle likes to think of himself as the boy who first announced that the emperor had no clothes, but what he is really doing is to require that all innovations of physical theory should satisfy the test of commonsense. He cannot see that special relativity is important precisely because it modifies Newtonian commonsense. In the twelfth century, I suspect he would have resisted the notion that the Earth is round in similar terms, by issuing a challenge that the round earthers should either complete the sentence “the bottom hemisphere (from which people will be decanted into nothingness) is that which . . .” or recant.

It may be asked why, if Dingle’s question has such a simple answer, it has not been provided before the appearance of the leading article two and a half years ago. The explanation is that Dingle has shifted his ground. In 1968, he seemed to be seeking to demonstrate that special relativity is internally inconsistent. Now he appears to accept that the theory is consistent, but says that its consequences are unacceptable.

It is also fair to say that the issue, through no fault of Dingle’s, has often

been confused by debates about situations where the accurate definition of inertial frames is crucial but difficult.

In all the circumstances, if there is a moral obligation undischarged, it rests with Dingle. He should acknowledge his error, so inform his small, devoted and in my view misguided band of supporters and then finally make his peace with the relativists.

JOHN MADDOX

London EC4

Scientific exchange

SIR,—A brief note by Wendy Barnaby in your issue of May 8 reports on a new exchange agreement between “Swedish and Russian Academies of Science” and comments that the “agreement is unique in that, unlike the others the Russians have with western countries, it allows for the host country to invite specific scientists by name for study visits rather than accepting the other country’s nominations.” This principle is extremely important in the normalisation of scientific relations with the Soviets, and I should therefore like to point out that the National Academy of Sciences of the USA sought and obtained such an “invitational” provision in its exchange agreement with the Academy of Sciences of the USSR in 1968 applicable to one-month lecture visits by outstanding scientists and broadly applicable to all categories of exchange scientists, including those spending up to a year at research, in our Inter-Academy Exchange Agreement for 1974 and 1975.

Although this invitational approach seemed to be alien to the Soviet Academy’s desires and practice with regard to a bilateral exchange agreement, the USSR Academy has increasingly acceded to the wishes of the National Academy of Sciences when it has requested consideration of the inclusion of individual named Soviet scientists whose presence is particularly desired at American universities.

LAWRENCE C. MITCHELL

National Academy of Sciences,
Washington 20418

From him and her

SIR,—We think he doth edit with ghastful grammar: “A year’s free subscription for he or she . . . Ed.” (May 8.)
Egad!

EDWARD B. ARMSTRONG

ELIZABETH B. ARMSTRONG

Narrabri, NSW, Australia

Oh woe. Even Fowler, often a friend in need, allows me no escape from this rebuke save (a) that “it is hard not to sympathise with the victims of this trap”, and (b) that Dickens did it, once. But the competition, in spite of all, is still open. Ed.

news and views

THE only statement about relativistic quantum electrodynamics which has consistently been made ever since 1927, when Dirac attempted to frame a modern theory of the motion of charged particles, is that it must be wrong and will break down. It is also the only feature of QED not yet verified.

At first, the doubts were because the ideas were spectacularly bizarre. Anti-matter was predicted at a time when the only known particles were the nucleus and the electron, and empty space became filled with the ghosts of uncreated particles of all energies. The diagrams developed by Feynman into a powerful tool to deal with the extremely complicated algebra of the theory enable one to visualise the kinds of process it calculates. A few of the infinite set which describe the effects of an external field on an electron (or muon) are shown in the figure, where the observable objects are the external field (X) and the particle as it enters and leaves the interaction region. All other particles (lines) and propagating fields (wavy lines) are evanescent. Only the sum of all terms can be measured. Fig. 1a represents the force experienced by a classical particle. In *b*, the particle emits a 'virtual' photon before the external interaction and reabsorbs it later, and in *c* the virtual photon turns for a short time into a ghost electron-positron pair. The effects of all strongly interacting particles from pi-mesons to the new ψ -particles are lumped together in *d*, which needs electron-positron annihilation data for its evaluation, in the absence of a theory of the strong interaction. The higher order terms such as *e* are so complex that the algebra as well as the arithmetic is done by computer.

In each order, it is necessary to remove the infinities which accompany the mass and charge of the idealised particles of the theory in a self-consistent way, so that the mass and charge which appear in the final expression are the values which an experiment would measure. The solution of the formidable technical difficulties of this procedure does not lessen the unease that infinite subtractions create, although the recent discovery that other theories may also be renormalisable and QED not just a unique accident of nature gives one more confidence (see John Taylor in *Nature*, 254, 560; 1975).

QED may not be philosophically or mathematically respectable, but does it

Measuring the effects of ghosts

from W. T. Toner

work? In parallel with an effort to which almost every notable twentieth century theorist has made a contribution there has been a determined attack by the experimentalists.

The first question is, do the processes actually take place at roughly the right rate? Most of the answers—all yes—have been given years ago but some have had to wait for the present generation of high energy accelerators. Frequently, the initial results have been contradictory. A recent example concerns the production of pairs of electrons and positrons by a heavy particle moving in the coulomb field of a nucleus. Jain *et al.* (*Phys. Rev. Lett.*, 32, 1460; 1974) claimed an anomalous result for pair production by muons, and are now contradicted by Fortney *et al.* (*Phys. Rev. Lett.*, 34, 907; 1975) who use a much more powerful and unambiguous technique to show that the theory works well in the related case of electromagnetic pair production by pi-mesons. (Not to be confused with production through strong interactions, which has a quite different behaviour.) It pays to choose the right technique.

In violent collisions at high energies, the dominant contribution often comes from one diagram which can then be tested under extreme conditions. Some

of the results from the SPEAR storage ring which tend to be overlooked in the excitement over the ψ particle are tests of the point-like behaviour of the idealised QED electron to distances of the order of 7×10^{-18} cm. It is noteworthy that satellite measurements of the Earth's magnetic field confirm that the inverse square law holds to distances of $\sim 10^{10}$ cm. There must be something right about a theory which spans such a range.

Our knowledge of higher order terms continues to improve. Bailey *et al.* (*Phys. Lett.*, 55B, 420; 1975) in the third of a beautiful series of experiments begun fifteen years ago at CERN have been able to increase the sensitivity of their measurements of the non-classical part of the magnetic moment of the muon to the point where the effects of Fig. 1d can be seen. Their result is $(g-2)/2 = (1,165,895 \pm 27) \times 10^{-9}$, where g is the Landé g -factor, equal to 2 for a classical muon. Excellent agreement with theory is obtained if Fig. 1d, $(73 \pm 10) \times 10^{-9}$, is included.

A second virtuoso experiment described in the same issue of *Phys. Lett.*, (55B, 411; 1975) by Bertin *et al.* is a measurement of the splitting between the 2s and 2p levels in helium ions where one muon has replaced the two electrons. Muons produced at the 600 MeV synchrocyclotron at CERN are brought to rest in a container of helium gas, surrounded by counters.

In a small fraction of the cases, the muons become trapped in the 2s level. On the signal that a muon has stopped in the gas, a Q-switched ruby laser is triggered. It in turn generates a brief pulse of infrared radiation from a tuneable dye laser. If the wavelength is just right, a $2s_{1/2}$ muon will be raised to the $2p_{3/2}$ level and will promptly drop down to the 1s ground state, emitting a tell-tale 8 kilovolt X ray as it goes. The laser was slowly scanned from 8,090 Å to 8,180 Å during the course of the experiment. The signal was contained in just 77 events in a narrow peak at 8,117 Å, out of nearly a million triggers.

Again, excellent agreement with theory is obtained, in this case giving the first precision measurement of the effect of Fig. 1f in atomic physics.

There is a small discrepancy with theory in muonic atoms where the nucleus is lead rather than helium. The theoretical difficulties now have a different aspect, as the power series is

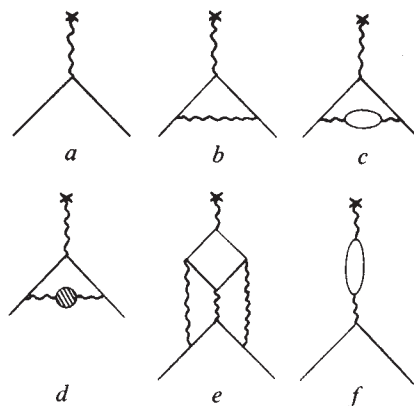


Fig. 1 Some of the diagrams representing the interaction of a particle with a field.

developed in Ze^2 rather than e^2 . Chen (*Phys. Rev. Lett.*, **34**, 341; 1974), now supported by Papatzakos (unpublished) claims to have resolved this discrepancy by including a previously neglected term. Others (*Phys. Rev. Lett.*, **34**, 399; 1974) disagree.

When do we stop? Two fundamental questions go completely unanswered as the theory now stands: Is it really in-

finite? Where did the muon come from? The key to both these questions may lie in the other interactions—weak, or even gravity. Some of the urgency behind the advocacy of the next generation of storage rings such as EPIC is due to the fact that weak interaction effects in QED processes should be clearly measurable with these machines.

Periodic cyclic AMP signals and cell differentiation

from J. D. Gross

THE cellular slime moulds, of which *Dictyostelium discoideum* is the best studied species, multiply as isolated individuals. When deprived of food they embark on the remarkable developmental phase of their life cycle. A few hours after starvation the cells begin to aggregate to a number of collecting points to form organised multicellular structures which ultimately become transformed into fruiting bodies made up of a cluster of spores held aloft by a thin column of vacuolated stalk cells. Two important observations on the events leading up to aggregation are reported in this issue of *Nature*. Shaffer (page 549) shows that cells exposed to a whiff of cyclic AMP themselves secrete cyclic AMP. This result puts the final seal on the evidence that cyclic AMP is the chemotactic signal by which the cells communicate during aggregation. Gerisch *et al.* (page 547) present evidence that these cyclic AMP signals are not only used to orient the amoebae but also play an important part in directing the physiological transformation of the cells from the actively multiplying state to aggregation competence.

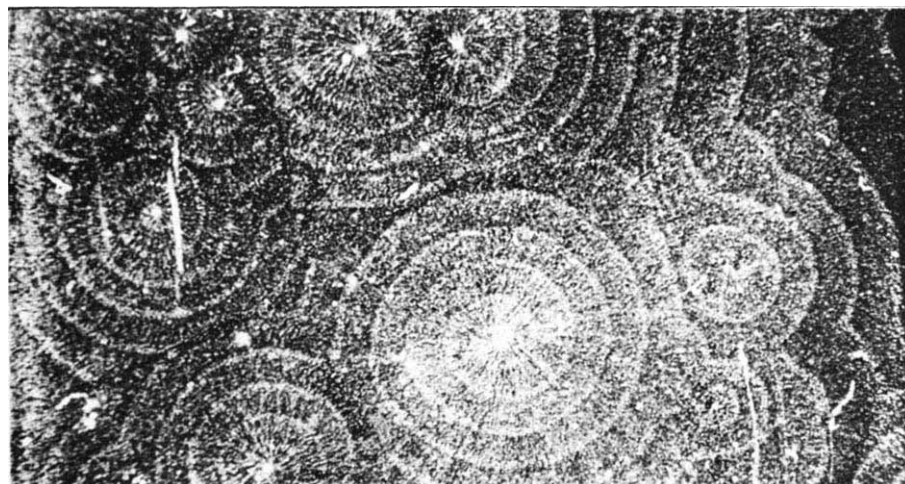
During the period of interphase between the onset of starvation and the appearance of clearly visible aggregation patterns the cells show an increase in responsiveness to cyclic AMP in chemotaxis tests such as those devised by Konijn and by Bonner (Bonner *et al.*, *Devl Biol.*, **20**, 72; 1969). Fully responsive cells have been shown to possess a potent surface-located phosphodiesterase that hydrolyses cyclic AMP, as well as a protein that binds cyclic AMP and is thought to be the receptor for the chemotactic response (Malchow and Gerisch, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2423; 1974). The cells can synthesise and secrete pulses of cyclic AMP in response to a signal, and some, the pacemakers for aggregation, actually secrete cyclic AMP spontaneously in a pulsatile fashion. The cells also display new cohesive properties associated with the appearance of sites (contact sites A) that permit the establishment of strong end-to-end contacts between cells (Beug *et al.*, *J. Cell Biol.*, **56**, 647; 1973). Once aggregation is under way waves of inward movement travel out from the pacemaker cells each time they release a pulse of

cyclic AMP (see figure), and these waves of inward-moving cells gradually become transformed into radial cell chains or 'streams' moving towards the centres. Evidently cells within range of a sufficient concentration of cyclic AMP respond not only by an inward movement step but also by relaying the signal. The outward propagation of the wave of movement requires that cells be refractory for some time after stimulation in order not to respond to the signal released by the cells distal to them. It is the central idea of a relayed pulsatile signal, first proposed by Shaffer (*Am. Nat.*, **91**, 19; 1957) that is firmly established by his latest results, as well as by similar observations of Roos *et al.* (*FEBS Lett.*, **53**, 139; 1975).

Work in several laboratories is now beginning to indicate that the various properties of aggregating cells do not develop autonomously but rather depend on interaction between the cells and the signals they emit. The evidence in this direction now obtained by Gerisch *et al.* is of two kinds. First, they find that if cells are permitted to enter stationary phase of growth rather than being taken at the exponential phase they fail to aggregate and that this developmental defect can be overcome by pulsing the cells with cyclic AMP for several hours to stimulate natural signalling. Second, they find that the time taken for 'normal' cells to reach the aggregation stage is decreased somewhat by this treatment whereas it is delayed substantially by applying cyclic AMP continuously. The latter treatment would be expected to interfere with pulsatile signalling set up spontaneously by the cells.

Darmon, Brachet and Pereira da Silva (*Proc. natn. Acad. Sci. U.S.A.*, in the press) have observed a similar acceleration of the development of normal cells by pulsing, as well as correction of the aggregation defect in certain mutants by the same treatment. A rather different approach has been taken by Alcantara, Bazill and Monk (unpublished) who analysed the observation that the time taken for cells to develop aggregation-competence increases markedly with decrease in cell density (Alcantara and Monk, *J. gen. Microbiol.*, **85**, 321; 1974), and found that the effect of low cell density could be overcome by the addition of purified cyclic AMP phosphodiesterase. This enzyme, by rapidly destroying extracellular cyclic AMP, presumably permits pulsatile signalling to be established at a time when the total activity of the enzyme in the dilute cell suspensions would otherwise be insufficient.

At first sight all these results seem to lead to the paradoxical conclusion that signalling between cells is itself required for the development of the ability to signal. This problem can be



Waves of chemotactic movement in aggregating cells of *D. discoideum* NC4, prepared by the method of Alcantara and Monk (*J. gen. Microbiol.*, **85**, 321; 1974). Most of the well-developed territories have spiral centres (see Durston, *J. theoret. Biol.*, **42**, 483). The photograph is approximately twice life size and was taken by M. Peacey and D. Trevan.

overcome by assuming that actively multiplying cells possess a weak capacity to detect and relay cyclic AMP signals, or develop this capacity during the first few hours of starvation in the absence of external signals, and that the ability to signal spontaneously only arises later (Robertson *et al.*, *Science*, **175**, 333; 1972). The first cells to emit cyclic AMP are apparently incapable of sustained signalling (Durstion, *Devl Biol.*, **37**, 225; 1974). But their spasmodic signals could be relayed over large distances, leading to increased responsiveness of the cells and to the gradual emergence of pacemakers able to emit regular signals. It is not yet known how the build-up of the components of the signalling system and of the cell contact mechanism is coupled to the signal response system, nor whether it involves an effect on gene expression. Coupling may occur by way of the intracellular oscillations of cyclic AMP levels (Goldbeter, *Nature*, **253**, 540; 1975) that probably accompany both spontaneous and induced signalling.

Eukaryote genes in *Escherichia coli*

from David Sherratt

SEQUENCE-SPECIFIC restriction endonucleases are now well established tools for inserting both eukaryote and prokaryote genes into plasmids, thus allowing gene organisation and expression to be analysed. The endonuclease *ecoRI* cleaves DNA about once every five to ten genes, generating short, self-complementary, cohesive ends. A number of plasmids and derivatives of bacteriophage λ contain a single *ecoRI* site; as they can still replicate in *E. coli* after other genes have been inserted at this site they provide suitable 'molecular vehicles' for cloning of inserted DNA sequences. The molecular vehicle is added to the required DNA and the mixture is cleaved with *ecoRI*, annealed, sealed with ligase and used to transform *E. coli* (or in the case of λ , to transfect *E. coli*). With plasmid vehicles, plasmid-borne drug resistance is usually used to select transformants and these are then analysed for the presence and nature of inserted DNA.

Clearly then, in order to isolate hybrids (or 'chimaeras') containing specific inserted sequences, one needs either a source of DNA highly enriched in those sequences, or alternatively a means of selecting or identifying the few bacteria containing the required genes from within a heterogeneous bacterial population. For the first approach, the amplified ribosomal genes of *Xenopus laevis* provide an ideal source of specific DNA which can

Control of ribosome synthesis

from A. A. Travers

A MAJOR activity of the bacterial cell is the synthesis of ribosomes. During normal exponential growth this process is finely regulated so that the rate of production of ribosomal RNA to a first approximation balances that of ribosomal proteins. Under these conditions the net synthesis of the principal macromolecular components of the ribosome is thus coordinated.

Another facet of the regulation of ribosome biosynthesis is the stringent response. When a stringent (*rel*⁺) strain is functionally starved of an amino acid the extent of rRNA and tRNA synthesis is immediately curtailed. In contrast relaxed (*rel*⁻) strains do not restrict stable RNA synthesis on amino acid deprivation. Another very early and apparently invariant manifestation of *rel*⁺ gene function during the stringent response is the accumulation of the guanine nucleotide ppGpp, itself a metabolic product of the ribosome. Its discoverers, Cashel and Gallant, proposed that this unusual nucleotide might act as a direct inhibitor of rRNA (and tRNA) synthesis. Consistent with this hypothesis is the observation that under conditions of partial amino acid starvation in a *rel*⁺ cell the rate of rRNA production is inversely correlated with the intracellular ppGpp concentration (Fiil, von Meyenburg and Friesen, *J. molec. Biol.*, **71**, 769; 1972).

Is the coordinate control of rRNA and r-protein synthesis that operates during normal growth maintained during the stringent response? Two recent complementary studies have produced strong evidence that to a large extent it is. Bennett and Maaloe (*J. molec. Biol.*, **90**, 541; 1974) showed

that like rRNA synthesis, the rate of r-protein synthesis relative to total protein synthesis is inversely correlated with the intracellular level of ppGpp. Similarly Dennis and Nomura (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3819; 1974) found that partial amino acid starvation resulted in the synthesis of rRNA and r-protein being more strongly inhibited than total protein synthesis. These same workers have now confirmed (*Nature*, **255**, 460; 1975) that this stringent control of r-protein synthesis reflects the regulation of the synthesis of mRNA coding for r-proteins. Thus as with rRNA synthesis stringency operates here at the level of transcription.

r-protein mRNA is the first class of messenger whose synthesis has been directly demonstrated to be under stringent control. Its regulation contrasts with that of *ø80*, *lac* and *trp* mRNA whose production is not inhibited in an amino acid starved *rel*⁺ cell. The simplest model for stringent control is thus to consider the synthesis of one class of mRNA being regulated in concert with rRNA and tRNA while the synthesis of a second class of mRNA is relatively unaffected. Any other mRNA species that are wholly or partially subject to stringency remain to be identified.

Although stringency is an important mode of regulating ribosome biosynthesis Dennis and Nomura hint that other controls may be superimposed on this rather coarse control because different ribosomal proteins are synthesised in vastly disproportionate amounts when the amino acid starvation becomes more severe. The authors suggest that this perhaps reflects an autogenous control of r-protein production.

be separated from the bulk DNA by its distinct buoyant density. It is therefore not surprising that this DNA was used in the work that led to the first report of replication and transcription of eukaryote genes in *E. coli* (Morrow *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743-1747; 1974). Another way of isolating specific eukaryote genes is to use the fact that some eukaryote mRNAs are relatively stable and are synthesised in large amounts by particular cells. Purification of these mRNA species followed by use of reverse transcriptase and DNA polymerase should allow synthesis of the relevant gene. Cohesive ends could be added *in vitro*

using deoxynucleotidyl terminal transferase (see, for example, Jackson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **59**, 2904-2909; 1972). Possible candidates for such synthesis are globin, immunoglobulin and crystallin genes. Another possibility for small genes, for example, those specifying polypeptide hormones, is complete *in vitro* synthesis of a nucleic acid that should specify the known amino acid sequence.

Using the alternative approach, Kedes *et al.* (in this issue of *Nature*, page 533) have now described an elegant method for the identification of bacterial clones containing specific DNA sequences from within a hetero-

geneous bacterial population transformed with a mixture of *eco*R1-cleaved plasmid and unfractionated eukaryote DNA. This method is potentially applicable to the isolation of bacterial clones containing any particular DNA sequences for which a specific labelled mRNA (or DNA) 'probe' is available; transcription of the inserted DNA in *E. coli* is not necessary. Kedes *et al.* suggest that this method should be able to identify clones containing genes which occur at frequencies as low as 10^{-6} , although their experiments describe the isolation of bacterial clones containing sea urchin histone genes which represent about 0.25% of total sea urchin DNA and which provide the template for large amounts of histone mRNA synthesis during embryonic cleavage.

Unfractionated sea urchin DNA was mixed with plasmid DNA (5:1 ratio) and the mixture was *eco*R1-cleaved, annealed, ligated and used to transform *E. coli*. About 5×10^{-4} of the plasmid-containing transformants were calculated to contain the histone genes and these were selectively enriched by an adaptation of the classical clonal enrichment technique of Cavalli-Sforza and Lederberg (*Genetics*, **41**, 367–381; 1956). Multiple samples (48, each containing 10^4 cells) were taken from the heterogeneous transformed population and serial three-fold dilutions of each were grown to stationary phase. Aliquots of all 48 samples of each dilution were pooled and assayed for the presence of histone genes by hybridisation to the labelled mRNA. The greatest dilution giving positive hybridisation was found; it should contain at least one and at most a few samples with cells containing histone genes. To identify these positive samples in 14 rather than 48 hybridisation assays, the 48 samples of this 'limit' dilution were arranged into a 6×8 grid and aliquots from every sample in each row were pooled and tested for their ability to hybridise to histone mRNA, as were aliquots from the samples down each column. In this way, the 'intercept' sample(s) was identified and the cells containing histone gene isolated from it by again arranging 96 cultures from single colonies into a 12×8 grid and identifying the intercept samples. By this technique, three independent clones containing histone sequences were isolated, each occurring at a frequency of about 5×10^{-4} , yet identified in about 40 hybridisation assays.

The hybrid plasmids from these bacterial clones containing histone genes were analysed in some detail. Most, if not all, of the sequences present in histone mRNA were present in two non-homologous *eco*R1 fragments having a total size of six kilobases (kb), the coding capacity of which is more

than sufficient to specify the structure of the histone proteins. Kedes *et al.* propose that it is this six-kilobase unit that is repeated some 470 times to give the known complement of histone genes in sea urchins. That this histone DNA is transcribed in *E. coli* minicells was also shown though the authors fail to enlighten us on the intriguing question of whether these genes are also translated in *E. coli*.

Kedes *et al.* point out that the possibility of isolating and purifying groups of genes that function concurrently at different stages of development now becomes a reality. It must be remembered, though, that considerable amounts of eukaryote DNA may never be transcribed and so cannot be identified by a mRNA probe. Nevertheless, this type of experiment, along with that described by Wensink *et al.* (*Cell*, **3**, 315–325; 1974), in which a number of individually cloned random segments of *Drosophila melanogaster* DNA were both analysed and mapped by *in situ* hybridisation should be invaluable in elucidating the nature of the eukaryote gene.

How mating algae agglutinate

from a Correspondent

CONTACTS between cells are important in such diverse phenomena as egg-sperm recognition, embryogenesis and tissue reorganisation. The complementary sites on cell surfaces that are recognised by other cells are being studied in a variety of systems including mammalian embryos and sponges. In many ways however there is a lot to be said for choosing simpler systems such as *Chlamydomonas* or yeast, the genetics of which can be handled conveniently. Crandall *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 26–29; 1974) have demonstrated the usefulness of the yeast *Hansenula wingei* in this regard. Intercellular recognition of opposite mating types leads to strong agglutination. The sex agglutinins are components of the cell wall of the yeast in the haploid state and can be solubilised in active form by proteolysis. They are mannan-protein complexes of widely heterodisperse molecular weight (15,000 to 10^6) and carbohydrate content (50–96%) whose synthesis is normally repressed in the nonagglutinative diploid state. The molecular basis of the strong interaction between complementary sex agglutinins of haploid cells of different mating types is unknown although both protein-carbohydrate and carbohydrate-carbohydrate complex formation could be involved since no agglutinin fraction has been shown to be quite free of residual protein.

The *Chlamydomonas* system has been exploited in similar fashion. *Chlamydomonas* is a unicellular flagellated green alga that differentiates in the vegetative phase into two mating types. These gametes are morphologically indistinguishable from undifferentiated vegetative cells or from each other. Nevertheless complementary surface sites are present since fully differentiated gametes from two mating types agglutinate when mixed, whereas homologous gametes do not. It is known that *Chlamydomonas* aggregation factors are glycoproteins present on the flagellar tips of the gametes. Glycoproteins isolated from gametes of one mating type can induce agglutination of the opposite mating type gametes. Homologous gametes or vegetative cells are not agglutinated. In other words during the process of gametogenesis the vegetative cells acquire at their cell surface glycoproteins that function in mating type specificity.

On the basis of sensitivity to purified enzymes of the agglutinative activity of gametic glycoproteins, Wiese and Haywood (*Am. J. Bot.*, **59**, 530–536; 1972) suggested that the active site of (+) gametes includes a sugar sequence and this sequence is recognised by protein(s) (perhaps also carrying a carbohydrate moiety) present in (–) gametes. The glycoproteins specific for mating type seem to be made *de novo* during gametogenesis and are rapidly (within an hour) renewed if they are artificially removed from the cells by trypsin. It is possible that extensive reorganisation of the cell surface occurs during gametogenesis. For instance simultaneously with the appearance of sex agglutinins, the gametes become agglutinable with concanavalin A (McLean and Brown, *Devl Biol.*, **36**, 279–285; 1974), whereas vegetative cells are poorly agglutinated. It is not clear whether the Con A binding glycoproteins are the same as the sex factors although McLean and Brown suggest that this is in fact the case. It is likely however that the Con A receptor sites (presumably mannose residues) are not involved alone in gamete recognition since simple sugars which inhibit Con A-induced agglutination do not affect intergametic agglutination. Of course it is still possible that gametic recognition requires an extended sugar sequence including mannose in the sex glycoproteins which, unlike the simpler binding requirement of Con A, is not competed for by simple sugars.

Some progress has been made recently on the protein component of the *Chlamydomonas* recognition system present in (–) gametes. McLean and Bosmann (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 310–313; 1975) have con-

sidered the earlier suggestion of Roseman (*Chem. Phys. Lipids*, **5**, 270–297; 1971) that recognition of cell surface sugar sequences may involve surface-located glycosyl transferases. These enzymes normally carry out reactions using oligosaccharide sequences as acceptors. The basic idea is that adhesiveness between cells is achieved by formation of an abortive or non-productive enzyme–substrate complex.

What McLean and Bosmann have done is to assess the ability of intact gametes of either mating type to utilise sugar nucleotide precursors in glycosyl transfer. It is argued that since the nucleotides do not permeate intact and viable cells, any transfer must involve participation by enzymes present at the cell surface. A wide variety of sugar transfer reactions involving galactose, mannose, N-acetylglucosamine, sialic acid, glucose and fucose were detected on *Chlamydomonas* gametes in this way. Transfer takes place on to endogenous glycoproteins presumably also located at the surface of the same or adjacent cells in positions accessible to the enzymes. McLean and Bosmann suggest that the glycosyl transferases and acceptors may be a contributing mechanism in gametic recognition and adhesion in *Chlamydomonas*. Thus, the sugar transfer reactions are enhanced several fold when gametes of opposite mating type are mixed. This interesting result indicates that one or both of the components of the putative recognition system (glycosyl transferase and its glycoprotein acceptor) are present in limited amount at the surface of gametes of either mating type and maximal complex formation involves adjacent cells of opposite mating type. It is assumed of course that such experiments measure surface-located reactions rather than simply reflecting damage to the cells by the conditions of incubation and the revealing of intracellular biosynthetic sites. Though this assumption is still unproven the preliminary results encourage the further use of the *Chlamydomonas* sexual system to define more precisely the recognition mechanisms operating between cells.

Lunar and terrestrial fields

from Peter J. Smith

It is far more difficult to obtain reliable ancient field intensities from rocks than it is to determine valid palaeomagnetic directions. Yet although the study of directions has proved to be the more fruitful on Earth, in the case of the Moon constraints on origin and evolution are far more likely to be derived from field magnitudes. The reason for

this is partly one of sampling, for it would be difficult to determine the shape of the ancient lunar field from the few, thinly distributed sites sampled so far even if all the rocks collected had been oriented. Moreover, as continental drift has no relevance to the Moon, one of the most important uses for terrestrial palaeodirectional data has no lunar equivalent. The determination of palaeointensities, on the other hand, requires neither orientation nor dense geographical coverage. It may be true that a complete interpretation of magnitude data requires information on the corresponding field shape, but the lack of such information is not unduly restricting. For a dipole field, for example, field intensities vary by a maximum factor of only two (equator to poles).

The problem with this analysis, however, is that it assumes that the field intensities are actually determinable, whereas in practice this is only so for a relatively small proportion of samples. Moreover, as Hoffman and Banerjee (*Earth planet. Sci. Lett.*, **25**, 331; 1975) have now discovered, there is a whole class of lunar rocks (with no terrestrial analogue) having properties which “could easily render any palaeointensity determination of little value”. When subjected to partial alternating frequency demagnetisation, certain crystalline rocks and breccias are found to have intensities of magnetisation which are non-reproducible at given a.f. fields; and in such cases the graph of natural remanent magnetisation against a.f. peak field has an unusual ‘zig-zag’ pattern. This behaviour has now been observed to varying degrees in many lunar rocks, and raises questions both as to its cause and as to the effects it is likely to have on the picture of the ancient field the rocks potentially provide.

As to cause, Hoffman and Banerjee have used both a.f. and thermal techniques to carry out a thorough study of lunar olivine basalt sample 15535—a rock in which the zig-zag behaviour is particularly marked. Perhaps the most surprising fact to emerge is that a substantial fraction of the total remanence in the basalt (that part responsible for the zig-zag pattern, in fact) is apparently confined to a single plane but is loosely pinned within that plane. When a.f. demagnetisation is carried out to a particular peak field, this ‘zig-zag remanence’ can take up any direction within the preferred plane; and when repeated demagnetisations are carried out at the same field, the zig-zag remanence will generally end up in a different direction each time. The remanence also varies in magnitude (hence the zig-zag), although neither peak a.f. fields of 1,000 oersted nor moderate temperatures are

sufficient to remove it completely.

The conclusion that Hoffman and Banerjee come to is that a high proportion of the basalt’s total remanence is carried by a few large disk-shaped multi-domain grains which are aligned by the local magnetic fabric. Whether the same can be said for all other lunar rocks exhibiting zig-zag behaviour is not yet known. But if the loose pinning of remanence to a plane is a common feature, it follows that conventional methods of palaeointensity determinations are likely to be useless. The Thellier method, for example, depends on a comparison of natural and thermoremanent magnetisations which have had a similar history of demagnetisation. If a large component of the natural magnetisation is ultimately undemagnetisable or behaves at all randomly under demagnetisation, the technique collapses.

Fortunately, this phenomenon is unknown in terrestrial rocks, although palaeointensity measurements are often precluded by a multitude of other problems. Nevertheless, using his new anhysteretic remanent magnetisation technique, Shaw has managed to obtain some interesting ancient field intensity data from the R_3 – N_3 polarity transition as recorded in the rocks of western Iceland (*Geophys. J.*, **40**, 345; 1975).

The results show that as the transition progressed from the fully reversed state to the fully normal state the field intensity generally decreased to very low values and then increased again. The lowest intensity recorded was equivalent to about 4% of the present value. But not all the intermediate dipole moments were low. When the geomagnetic pole reached the geographic equator it apparently remained there for a time while the dipole moment rose smoothly to a value about 21% higher than even the present fully ‘normal’ value, and then decreased equally smoothly to the very low values preceding the increase to the N_3 state.

Shaw’s interpretation of this remarkable phenomenon is that the Earth’s magnetic field could well have a third metastable state (intermediate, in addition to normal and reversed) in which the pole is temporarily fixed and the dipole moment is large. In the short periods (transitions) between metastable states, by contrast, the pole position changes rapidly while the dipole moment is small. Because the intermediate state is likely to be short, examples will be difficult to detect palaeomagnetically; but if confirmed as a general feature of the geomagnetic field, it will clearly place an additional constraint on theories of field origin. It will be a long time before such fine points can be made in connection with the lunar field. □

Progress on penicillinase

from David R. Thatcher

A workshop was held at the University of Newcastle upon Tyne from April 16 to 18 to discuss the biochemistry of bacterial penicillinases.

PENICILLINASES, or as they are now known, β -lactamases, are enzymes which specifically deactivate penicillin by hydrolysis of the β -lactam ring. Antibiotic resistance in a number of pathogenic bacterial strains has been conclusively linked with the production of these enzymes. In recent years transmissible plasmids or R factors carrying β -lactamase genes have been responsible for the rapid spread of resistance among organisms previously sensitive to penicillin. Penicillinases are therefore of immense medical importance and a detailed knowledge of their structures and mechanisms will be crucial in the development of new approaches to penicillin chemotherapy.

The present uses of β -lactamases in pharmaceutical research were outlined by G. W. Ross (Glaxo, Greenford). He pointed out that as new prospective antibiotics are synthesised it is essential that they be exposed to as wide a spectrum of naturally occurring β -lactamases as possible. During the past few years the interest of the drug companies has shifted from the gram-positive producers like *Bacillus cereus*, to gram-negative producers like *Pseudomonas aeruginosa* and *Escherichia coli*, as the greater medical significance of the latter group and their R factors was realised. β -Lactamases from a large number of gram-negative sources have been characterised at Glaxo, using immunological techniques and a new isoelectric focusing method. Several of these enzymes were purified to homogeneity using an interesting single-step QAE-Sephadex column chromatography method. J. W. Dale (University of London) reviewed the range of penicillinase types determined by R factors, including a fascinating class of dimeric β -lactamases having a high specificity for oxacillin.

It is becoming increasingly apparent, however, that any fundamental advances in antibiotic design will be based on our physico-chemical understanding of the interaction of the drug with the target enzyme (in this case the cell wall transpeptidase) and any specific resistance mechanisms (in this case, penicillinase). Structural studies at the moment are still governed by the availability of large quantities of pure enzyme and consequently most of our information is confined to the β -lactamases of gram-positive super-producing strains of little medical importance.

Complete amino acid sequence data are available on the enzymes from *Staphylococcus aureus* and *Bacillus licheniformis* with partial data on the sequence of the β -lactamase I of *Bacillus cereus* (80%) and preliminary data on the *E. coli* TEM enzyme. R. P. Ambler (University of Edinburgh) explained how the three gram-positive sequences known show a high degree of similarity to each other whereas the fragmentary data available on the *E. coli* TEM enzyme show little, if any, evidence for homology.

X-ray crystallographic analysis of two enzymes, the *E. coli* TEM enzyme (J. R. Knox, University of Connecticut) and the *S. aureus* penicillinase (D. W. Green, University of Edinburgh) has commenced and L. Sawyer and J. Moulton (University of Edinburgh) presented a report of progress made up to now. It is hoped that low resolution structures will soon be completed for both enzymes.

The conformation of the *S. aureus* penicillinase in solution has been examined by difference spectroscopy, optical rotatory dispersion and circular dichroism (R. H. Pain, University of Newcastle upon Tyne). Gradually unfolding the protein in increasing concentrations of guanidine hydrochloride revealed a partially unfolded state(s) which although retaining all α -helical structure, was enzymologically inactive and appeared, on the basis of optical absorption and flow properties, to be expanded, with the 13 tyrosine residues fully exposed to solvent.

By analysing the equilibrium constants of the transitions between the native, partially unfolded and fully unfolded states, Pain and co-workers have been able to calculate the free energies of the two denatured states, with respect to the native state. These values are much lower than, for example, those found in the denaturation of ribonuclease or lysozyme. *S. aureus* penicillinase could in fact be described as a 'floppy' molecule and Pain has calculated that in solution up to 3% of the enzyme molecules are in the partially unfolded state(s). Conformational flexibility is becoming a characteristic property of β -lactamases or, more specifically, conformational changes provide the best explanation of a number of unusual penicillinase properties.

The search for residues involved in the catalytic site of β -lactamases continues without marked success. S. G. Waley (University of Oxford) presented k_{CAT}/K_M against pH plots for the hydrolysis of benzylpenicillin by the β -lactamase of *B. cereus*. The usual bell-shaped plots appeared and these results were explained on the basis of the ionisation of groups in the catalytic centre (pK_a s of 5.5 and 8.6) affecting substrate binding. The group with a pK_a of 5.5

could either be a carboxyl or an imidazole side chain, Waley tentatively favouring the former on the basis of inactivation studies using Woodward's reagent. Waley also described a number of elegant inhibition experiments using penilloates (decarboxylation products of penicilloic acid) and showed that these compounds were much better inhibitors than the natural penicilloic acid products.

Stopped-flow spectrophotometric data (S. Halford, University of Bristol) using the *S. aureus* enzyme and a chromogenic substrate showed that in this case the bell-shaped plot of k_{CAT} against pH could arise by the change of one rate-determining step to another with pH. Rapid reaction kinetics resolved the reaction into three phases, the formation of (ES), conversion of (ES) to (EP) and the decomposition of (EP) to the free enzyme and product. Below pH 6.0 the reaction rate is limited by the conversion of (ES) to (EP) and above pH 6.0 by the decomposition of (EP).

Using a pH indicator in the assay system, Halford was able to show that proton release accompanied the (ES) to (EP) transition; a result which favours a general acid-base catalytic mechanism rather than a hypothesis involving the formation of a covalent acyl intermediate. If the release of this proton is involved in the rate-limiting decomposition of (ES), a primary isotope effect should be observed when the reaction is performed in D_2O . As no such effect was observed the rate of conversion of (ES) to (EP) probably also involves a rate-limiting conformational change after proton release.

R. Virden (University of Newcastle upon Tyne) and co-workers have been studying the effect of quinacillin inhibition on *S. aureus* penicillinase. This penicillin analogue is a substrate, albeit a poor one, yet it progressively inactivates the enzyme and on subsequent dilution into benzylpenicillin solutions the inhibited enzyme only slowly regains normal enzymic activity.

Kinetic analysis of this reversible inhibition has been performed and was explained in terms of quinacillin binding preferentially to an inactive conformation of the enzyme. Denaturation during quinacillin hydrolysis yields a covalent enzyme-substrate complex. Chemical analysis of this complex has not yet shown whether or not quinacillin is bound at a single site.

High resolution structural data on a β -lactamase will be invaluable in the interpretation of many of the kinetic and chemical modification data. Apart from the potential benefits to medicine, it looks as if future research on penicillinases will make many valuable contributions to our understanding of enzyme function in general.

articles

Lead and strontium isotopes in post-glacial basalts from Iceland

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We report here our Pb and Sr isotopic study of some post-glacial basalts (less than 12,000 yr old) from the southern part of Iceland, and use our new data to evaluate various proposed models for the genesis of volcanic rocks on Iceland. The interpretation for Icelandic rocks is also extended to the genesis of volcanic rocks on other oceanic islands of the world.

SAMPLES of basalts from Iceland (locations shown in Fig. 1) have been analysed at the NASA Johnson Space Center. Lead isotope compositions (Table 1) were analysed using the silica gel method, and the $^{206}\text{Pb}/^{208}\text{Pb}$ ratio of NBS 982 equal atom standard was routinely analysed as a monitor for the mass fractionation correction. During the study, our silica gel gave -0.12% mass fractionation per mass unit difference. CIT standard Pb determined by us gives $^{206}\text{Pb}/^{204}\text{Pb} = 16.637 \pm 0.010$, $^{207}\text{Pb}/^{204}\text{Pb} = 15.482 \pm 0.010$ and $^{208}\text{Pb}/^{204}\text{Pb} = 36.300 \pm 0.030$ (2σ). These values agree well with 'absolute values' reported by Catanzaro¹. The uncertainties (2σ) are believed to be better than $\pm 0.1\%$ for $^{206}\text{Pb}/^{204}\text{Pb}$, $\pm 0.15\%$ for $^{207}\text{Pb}/^{204}\text{Pb}$ and $\pm 0.2\%$ for $^{208}\text{Pb}/^{204}\text{Pb}$. Analysis of Sr compositions gives the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of NBS 987 standard as 0.71025 ± 2 ($2\sigma_m$, based on 15 replicate analyses with a total range of 0.00012). The uncertainties quoted in Table 1 represent in-run precision. No replicate analysis was made.

Basic data

The isotopic compositions of Pb and Sr, and the concentrations of U, Th and Pb are presented in Table 1. Welke *et al.*² reported lead isotope data for some Icelandic igneous rocks of Miocene to recent age and found that the rocks could be divided into two groups based on the isotopic compositions. The less radiogenic group (Group I) includes older (7–16 Myr) rocks from eastern, northern and north-western Iceland and post-glacial rocks from the Northern Neovolcanic Zone (Fig. 1); the more radiogenic group (Group II) consists of younger (< 3 Myr to recent) rocks from western, south-western and central Iceland, including Surtsey. We have recalculated their $^{206}\text{Pb}/^{204}\text{Pb}$ data to 'absolute' ratios based on their Broken Hill galena lead standard. The new values are plotted along the $^{206}\text{Pb}/^{204}\text{Pb}$ axis of Fig. 2. The reason for doing so is that their $^{207}\text{Pb}/^{204}\text{Pb}$ values are not accurate enough to be useful and the present technique for acquiring high quality data enables us to measure more accurate $^{207}\text{Pb}/^{204}\text{Pb}$ ratios.

Consequently, we can determine more accurately the $\Delta^{207}\text{Pb}/\Delta^{206}\text{Pb}$ slope which is vital in data interpretation.

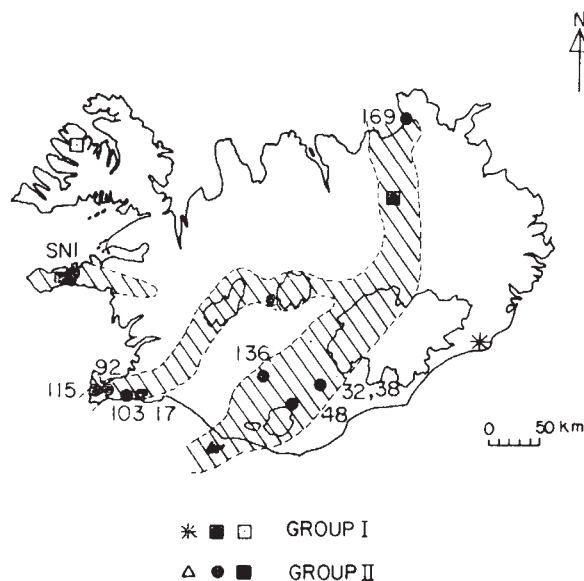
The regression line in Fig. 2 was calculated using the York method³. For replicate analyses, such as I-48 and I-103, the average values were used. The slope of this regression line is 0.09 ± 0.02 (2σ) which is similar to that for other oceanic islands⁴. For example, the slope ($\Delta^{207}\text{Pb}/\Delta^{206}\text{Pb}$) for the Canary Islands samples is 0.110 ± 0.014 (2σ), with a total range of $^{206}\text{Pb}/^{204}\text{Pb}$ variation about 5%.

The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the rocks (I-92, I-17, I-103 and I-115) from the Reykjanes Peninsula (Central Neovolcanic Zone) have a narrow range of 0.70305 to 0.70315. Hart *et al.*⁵ also reported Sr isotopic compositions for four rocks from the same general area and they range from 0.70301 to 0.70308 (IC-47, IC-62, IC-58 and R-11), after making an adjustment of NBS 987 Sr standard value.

A sample from the Snaefellsnes Zone (SN-1) has a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70345 ± 5 , substantially greater than values from the Central Neovolcanic Zone. Hart *et al.*⁵ and O'Nions and Pankhurst⁶ also reported larger values (about 0.70330) for the Snaefellsnes rocks.

The samples from Eldgja (I-48) and Hekla (I-136A) of the Eastern Volcanic Zone (EVZ) also have larger $^{87}\text{Sr}/^{86}\text{Sr}$ ratios than the volcanic rocks from the Central Neovolcanic Zone.

Fig. 1 Sampling localities. The localities of samples analysed by Welke *et al.*² are also shown.



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Table 1 Lead and strontium isotopic compositions and U, Th and Pb concentrations in Iceland basalts

Sample no.	Location	Concentration (p.p.m.)			Atomic ratio					$^{87}\text{Sr}/^{86}\text{Sr}^*$
		U	Th	Pb	$^{232}\text{Th}/^{238}\text{U}$	$^{238}\text{U}/^{204}\text{Pb}$	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	
I-48	Eldgja lava, Eldgja	0.553	1.84	1.58	3.37	26.1	19.260	15.558	38.925	0.70336 $\pm$ 4
SN-1	Berserkjahraun flow, Snaefellsnes	0.319	1.03	1.04	3.27	22.8	19.249	15.551	38.908	0.70345 $\pm$ 5
I-136A	1913 Lamfitjarhraun eruption north of Hekla	0.584	1.82	1.61	3.14	26.8	19.110	15.521	38.697	0.70323 $\pm$ 3
I-92	Arnarsetur, Reykjanes Peninsula	0.148	0.433	0.677	2.97	16.2	19.128	15.536	38.725	0.70314 $\pm$ 3
I-17	Krysuvikurhraun, Reykjanes Peninsula	—	0.445	0.576	—	—	19.008	15.510	38.516	0.70315 $\pm$ 5
I-103	Near Isolfsskali, Reykjanes Peninsula	0.0844	—	0.414	—	14.9	19.012	15.506	38.506	0.70305 $\pm$ 6
I-115	Near Langholl, Reykjanes Peninsula	0.0644	—	0.475	—	9.99	18.761	15.487	38.307	0.70309 $\pm$ 6
I-32	Laki	0.304	0.985	1.01	3.28	21.9	18.759	15.486	38.304	0.70314 $\pm$ 5
I-38	Laki	0.594	1.87	1.30	3.19	33.4	18.751	15.503	38.337	0.70322 $\pm$ 4
I-169	Leirhafnarfjall						18.474	15.476	38.204	0.70314 $\pm$ 5
							18.472	14.471	38.192	
							18.478	15.476	38.222	
							18.466	15.485	38.178	

*NBS-SRM987 value = $0.71025 \pm 2(2\sigma_m)$

All errors quoted represent in-run precision $2\sigma_m$.

O'Nions and Gronvold⁷ also reported bigger ratios for ice-landites from both Torfajökull and Hekla areas.

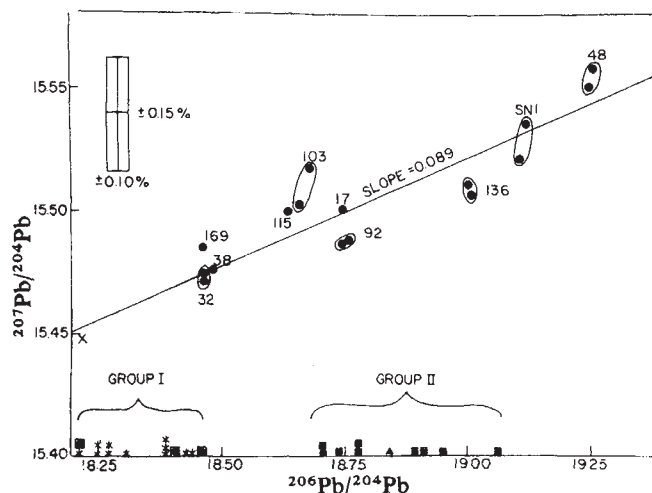
Two samples from the Laki area (I-32, I-38) have similar Pb but perhaps slightly different Sr isotopic compositions. Their U and Th concentrations are, however, different by a factor of two.

Our Sr data are in close agreement with those of Hart *et al.*⁵ in all comparable cases. Hart *et al.*⁵ and O'Nions and Pankhurst⁶ pointed out that the values reported by O'Nions, Pankhurst and others⁸⁻⁹ are systematically greater by about 0.00010 than those of Hart *et al.* after the adjustment of Sr standard values was made.

The data in Table 1 are arranged in a general trend of decreasing radiogenic nature of Pb isotopes. Most of the Sr isotope ratios (except I-38) follow the same trend. But, it seems that the radiogenic nature of Sr isotopes also parallels the concentrations of U and Th in the rocks, for example, I-48, SN-1, I-136A and I-38 have larger $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, as well as U and Th contents, than others.

A very uniform value of $^{87}\text{Sr}/^{86}\text{Sr}$ was emphasised by O'Nions and Gronvold⁷ and Hart *et al.*⁵ for most of the post-glacial basalts from Iceland. In contrast, our present data show that the basalts with alkali affinity from the flanking zones have a more radiogenic nature.

Fig. 2 A $^{207}\text{Pb}/^{204}\text{Pb}$ – $^{206}\text{Pb}/^{204}\text{Pb}$ plot of our new data. $^{206}\text{Pb}/^{204}\text{Pb}$ values for samples of Welke *et al.*² were corrected to absolute values and plotted along the $^{206}\text{Pb}/^{204}\text{Pb}$ axis for comparison. Symbols used are the same as in Fig. 1. A basalt from the Reykjanes Ridge (x) is also plotted for reference.



O'Nions and Pankhurst⁸ observed a secular variation in the Sr isotope composition of Icelandic volcanic rocks. Older rocks are generally more radiogenic than the younger rocks. This contradicts the trend found by Welke *et al.*² from their Pb isotope study.

Existing models

The existing model interpretations for Icelandic Pb and Sr isotopic data may be grouped into two categories: disequilibrium (Rayleigh) partial melting of a homogeneous mantle source or crustal contamination of a partial melt from a homogeneous mantle source; and equilibrium partial melting in various domains of heterogeneous mantle source, with mixing of the resulted melts permissible.

The Pb isotopic compositions of Group I rocks (older) were regarded by Welke *et al.*² as representing the isotopic character of the mantle source. They thought that the younger magmas of Group II might have picked up radiogenic leads by highly selective wall rock reactions on their way to the surface. The contaminants (wall rocks) were believed to be slightly older yet highly differentiated rocks, such as granophyre or obsidian underlying the post-glacial volcanic piles. To explain the large difference in $^{206}\text{Pb}/^{204}\text{Pb}$ ratios but very narrow range of $^{207}\text{Pb}/^{204}\text{Pb}$ ratios between the two groups, they suggest that the acquired radiogenic lead must be relatively young (~20–30 Myr). Furthermore, the possibility of contamination with ancient lead, as commonly observed in continental areas, is totally ruled out.

O'Nions and Pankhurst⁸ observed secular variation of the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios among the Icelandic volcanic rocks. The ratios decrease progressively from 0.7036 at 15 Myr BP to 0.7033 at 2 Myr BP (E and A standard = 0.70815 ± 1). They proposed three possible ways of interpretation:

- (1) Disequilibrium (Rayleigh) partial melting of a homogeneous mantle source. During partial melting process, there is no isotopic equilibration between the liquid and the residual solid phases. Amphibole and phlogopite which have more radiogenic Sr and higher Rb/Sr ratios preferentially enter the early melt. The secular variation may be caused by Rayleigh melting of a single source through time (considered unlikely⁸ regarding to the upwelling and spreading rates of the mantle materials); or by increase in degree of partial melting of continuously replenished mantle (thus constant mantle composition) over the past 15 Myr.
- (2) Partial melting of heterogeneous mantle sources.
- (3) Mixing of two or more mantle sources and progressive change of the mixing ratio with time. This interpretation contains two alternative possibilities: Contamination (or mixing)

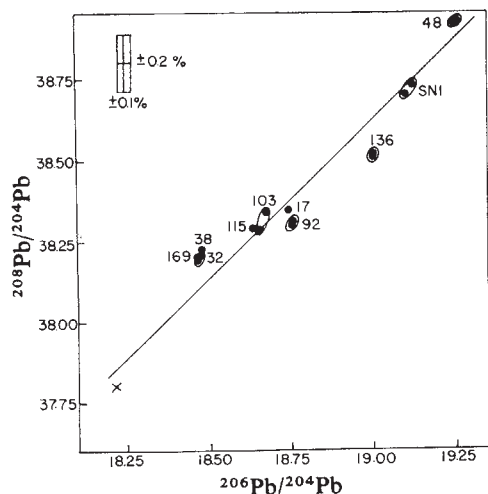


Fig. 3 A $^{208}\text{Pb}/^{204}\text{Pb}$ - $^{206}\text{Pb}/^{204}\text{Pb}$ plot of our new data. Symbols used are as in Fig. 1. A basalt from the Reykjanes Ridge (x) is also plotted for reference.

of upwelling normal mid-ocean ridge basalt magmas through a segment of mantle enriched in incompatible elements and radiogenic ^{87}Sr , with the contamination effect decreasing with time; or mixing of an upwelling mantle plume (relatively enriched in incompatible elements and radiogenic ^{87}Sr) with low velocity zone material, with the plume activity decreasing with time since 15 Myr BP.

Implications of new data

The linear array of our Pb isotopic data, which yields a slope of 0.09 ± 0.02 (2σ), argues against the interpretation of Welke *et al.* that younger magmas were contaminated by older Icelandic volcanics, because the regression line produced by such contamination would probably have a shallow slope² of 0.046 due to recent radiogenic lead growth from ^{235}U and ^{238}U . Moreover, our data suggest that if Rayleigh melting in a homogeneous mantle or equilibrium partial melting in a heterogeneous mantle was responsible for the observed Pb isotopic variation among the Icelandic samples, the age for the source rock is about $1,500 \pm 500$ (2σ) Myr.

Wall rock reaction and Rayleigh melting were considered to enrich the radiogenic daughters in the melts, thus the general increase of $^{206}\text{Pb}/^{204}\text{Pb}$ ratios accompanied by the decrease of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the younger magmas⁸ indicates that the sub-Icelandic mantle is not a simple system. Our data cannot be satisfactorily explained in terms of a homogeneous mantle source. Furthermore, the $^{87}\text{Sr}/^{86}\text{Sr}$ values for alkali basalts from Surtsey and Heimaey are not higher than those of olivine tholeiites from the Central Neovolcanic Zone⁹. This is also not consistent with what we expect from the Rayleigh melting; the problem is discussed in detail elsewhere (ref. 10 and S.-S.S., M. Tatsumoto and J.-G. Schilling, to be published). This leaves models which involve heterogeneous mantle sources for the Icelandic magmas.

A mixing model in which upwelling mid-ocean ridge basalt magmas mix with the overlying mantle enriched in incompatible elements is difficult to reconcile with our new data. Suppose that part of mantle is characterised by large $^{87}\text{Sr}/^{86}\text{Sr}$ and small $^{206}\text{Pb}/^{204}\text{Pb}$ as suggested by the older Icelandic volcanics. The decrease of involvement of this mantle domain in the magma generation with time should reduce both observed $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ ratios, because the $^{206}\text{Pb}/^{204}\text{Pb}$ ratios of normal ridge basalts are low (ref. 4 and unpublished work of S.-S.S. and others) (~ 18.20 for basalts from the Reykjanes Ridge.)

In contrast, most younger Icelandic rocks have greater $^{206}\text{Pb}/^{204}\text{Pb}$ ratios than the older rocks. To avoid this problem, the overlying mantle must be heterogeneous in Pb isotopic

composition. It requires a very radiogenic character for the southern part of Iceland, except for the Laki samples which seem to be similar to the mantle that produced older Icelandic magmas.

Our choice of model is strongly influenced by a Pb isotope study of the Reykjanes ridge basalts (unpublished work by S.-S.S. and others). In this model, mixing of the Icelandic mantle and the normal mid-ocean ridge mantle (the low velocity zone?) along the Reykjanes Ridge is strongly suggested. This is somewhat similar to the mantle plume-low velocity zone mixing model proposed by Schilling¹¹. We consider that the Icelandic magmas were probably not derived from a pure mantle plume component. Instead, they were formed by a mixing of the plume source and the material of low velocity zone as suggested by O'Nions and Pankhurst⁸. The involvement of the plume component is believed to have decreased with time since 15 Myr BP. Good evidence to support this mixing model is that all Pb isotope data from Iceland and the Reykjanes Ridge form a linear array in both $^{207}\text{Pb}/^{204}\text{Pb}$ - $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ plots.

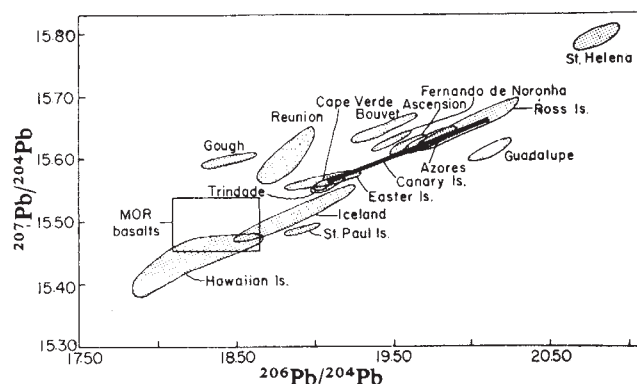
Implications of our model

If the decrease of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in younger magmas⁸ is used as an indication of decreasing plume activity, then both our data and those of Welke *et al.* suggest that the plume component itself is not homogeneous in Pb isotopic composition. Our Sr data also indicate that the post-glacial magmas do not have uniform $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. In general the recent 'plume' material that upwells in the southern part of Iceland seems to be more radiogenic in Pb isotopic composition than that in northern Iceland. In the present interpretation, the basalts from Snæfellsnes, Eldgja and Hekla are explained as being derived from less mixed (less diluted) or unmixed plume sources.

Being limited by the inherent analytical uncertainty in the Pb isotope analysis and the availability of the Pb data for Icelandic rocks, it is difficult, if not impossible, to characterise the Pb (or Sr) isotopic composition for the plume component at this time. Consequently, the evolutionary history of the plume source cannot be well defined in terms of any model such as the two-stage model (see, for example, refs 4 and 12).

The Icelandic Pb isotopic study has a profound impact on the interpretation of Pb isotopic data of the volcanic rocks in oceanic islands. As shown in Fig. 4, most oceanic island leads have regression lines pointing toward the field of mid-ocean ridge basalts^{4,7}. These data were previously interpreted in terms of a modified two-stage model and a mantle-mixing model⁴. Since most of the oceanic islands including Iceland (Fig. 4) have been suggested as the locations of plume uprising¹³, it is possible that some of the regression lines of the oceanic islands also represent mixing lines similar to the case of Iceland. Consequently, they do not have an age significance as in a two-stage model. But our present state of knowledge does not rule out the possibility of a two-stage model.

Fig. 4 A general picture of the Pb isotopic compositions for the oceanic islands. Data from Sun⁴ and unpublished data.



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Kinky helix

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DNA in chromatin is highly folded. Is it kinked? And does it kink in other situations?

CHROMATIN is the name given to chromosomal material extracted from the nuclei of cells of higher organisms. It consists mainly of DNA and a set of small rather basic proteins called histones. Other proteins and RNA are present in lesser amounts (see for example ref. 1). Early X-ray work (for review see ref. 2) suggested that there was a structure in chromatin which repeated at intervals of about 100 Å. More recent work using nucleases^{3,4} has shown that the DNA in chromatin exists in some regular fold which repeats every 200 base pairs, the best value currently being 205 ± 15 base pairs⁵.

The most cogent model for chromatin has been put forward by Kornberg⁶ who suggested that the basic structure consists of a string of beads each containing two each

of the four major histones, each bead being associated with about 200 base pairs of DNA. Linear arrangements of beads (in a partly extended form) were first seen in the electron microscope by Olins and Olins⁷ and called by them ν -bodies. The exact diameter of a bead in the wet state is rather uncertain but it is probably in the region of 100 Å. Kornberg's model suggested that DNA, when associated with histone, is folded to about one-seventh of its length. This is the value deduced by Griffith⁸ from electron micrographs of the mini-chromosome of the virus SV40. A similar value has been obtained by Oudet *et al.*⁹ from measurements on adenovirus 2. Other compact models have been proposed by van Holde *et al.*¹⁰ and Baldwin *et al.*¹¹.

Thus the DNA in chromatin, even at this first level of structure, must be folded considerably since its length is contracted to about one-seventh. Moreover, the basic repeat of 200 base pairs (which is 680 Å long in the B form of DNA) must be folded into a fairly limited space having the dimensions of about 100 Å³ (ref. 6).

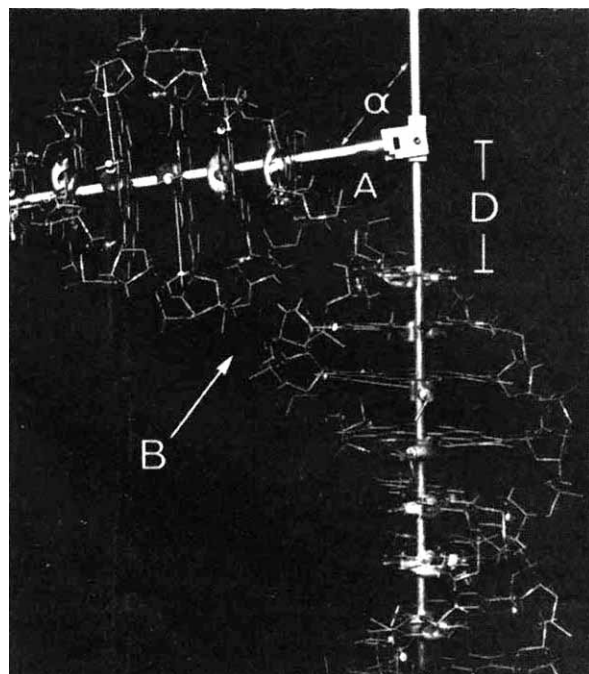
We have found it very difficult to estimate just how much energy is required to bend DNA "smoothly" to a small radius of curvature, say 30–50 Å, bearing in mind that these numbers are not many times greater than the diameter of the DNA double helix, which is about 20 Å, and that bending a helix destroys its symmetry. We have formed the impression that the energy might be rather high. We therefore asked ourselves whether the folded DNA may consist of relatively straight stretches joined by large kinks. This paper describes a certain type of kink which can be built rather nicely and has interesting properties.

The stereochemistry of a kink

No doubt other types of kink could be built, but we have concentrated on one special type which we consider to be rather plausible. We have assumed that all the base pairs of the double helix are left intact (so that no energy is lost by unpairing them), that the straight parts of the DNA on each side of the kink remain in the normal B form, but that at the kink one base pair is completely unstacked from the adjacent one. Thus at each kink the energy of stacking of one base pair on another is lost. Naturally all bond distances and angles (including dihedral angles) have to be stereochemically acceptable.

We find that, given these assumptions, one can convincingly build a neat kink, having a large angle of kink, in one way only; or, more strictly, in a family of ways all very similar to each other. The double helix is bent towards the side of the minor groove. This can be seen in the photograph of one such model shown in Fig. 1.

Fig. 1 General view of a model of a kink, taken from the side. For this model $d = 0$, $\alpha = 98^\circ$, $D = 8$ Å and $\theta = 23^\circ$ (see text). The two short lengths of backbone, connecting the two stretches of straight helix, can be seen at A. The region of van der Waals' contacts between backbones, which limit the kink angle α , is near B.



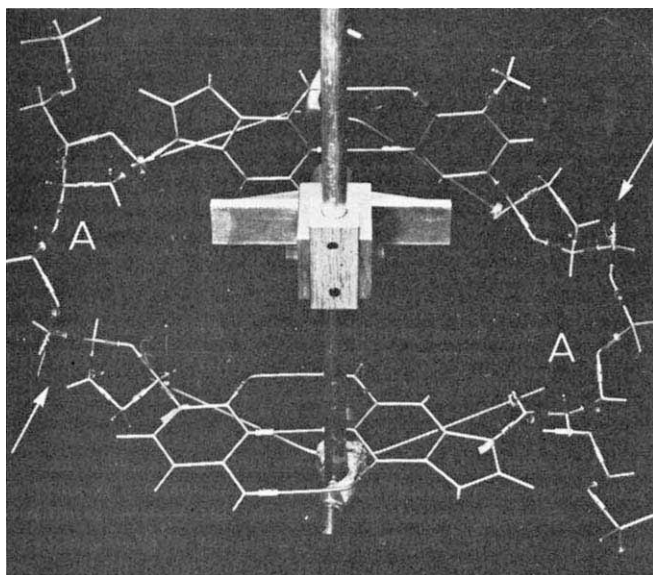


Fig. 2 View of part of the model of Fig. 1 taken approximately looking down the pseudo-dyad. It shows two base pairs, one on either side of the kink. The rest of the model has been blanked out for easier viewing. The two arrows point to the $C_4'-C_5'$ bonds at which the chain conformation is changed by kinking—see Fig. 3. The letters A correspond to the region marked A in Fig. 1.

The structure can be built with an approximation to a dyad axis passing through the kink, though we cannot see any strong reason why such symmetry is essential in chromatin. A partial view looking along the pseudo-dyad is shown in Fig. 2.

To our surprise the configuration of the backbone at the kink can be made similar to that of the normal backbone of the B form except that the conformation at the $C_4'-C_5'$ bond in the sugar is rotated 120° about this bond, going from one of the possible staggered configurations to another one as shown in Fig. 3. (We have arbitrarily kept the same pucker of the sugar ring as is found in the B form of DNA.)

The structure of the backbone at the kink is not one of the preferred configurations that have been listed^{12,13} since these involve a weak $CH \cdots O$ close contact between the hydrogen attached to either C_6 of a pyrimidine or C_8 of a purine and the O_5' of the sugar, and by its very nature the type of kink we have assumed cannot have this at every position. In our model this contact is absent for one base of each of the base pairs immediately adjacent to the kink. As explained above, however, the backbone configuration is sufficiently close to that of the B form of DNA (except for the torsion angle about $C_4'-C_5'$) that we feel it is acceptable stereochemically.

The nature of the base pairs on either side of the kink is immaterial to the model though presumably the energy required to unstack these base pairs will depend to some extent on their composition. We have found it difficult to estimate the free energy involved. It is probably a few kilocalories. It is obviously desirable that this figure should be determined as accurately as possible since the ease of making kinks depends on just how big it is. Another important question is how much a DNA double helix can be bent before it kinks. If we denote the mean curvature by κ (where κ equals the reciprocal of the radius of curvature) then we would expect the energy of deformation of a uniformly bent helix, per unit length, to increase at least as fast as κ^2 . For a kinked helix, on the other hand, this energy increases only as κ . In this case κ is the mean 'curvature' of the segmented double helix which is proportional to the number of kinks per unit length. Thus, as is

intuitively obvious, at small κ the double helix will bend, while at large κ it will kink. The value we should like to know is the radius of curvature at which it changes from bending to kinking.

There is probably an appreciable activation energy to the process of making a kink since the $C_4'-C_5'$ bond must pass through the eclipsed configuration. For this reason we consider kinks of this type with a kink angle of about half the full 100° to be unlikely.

Common features of the family

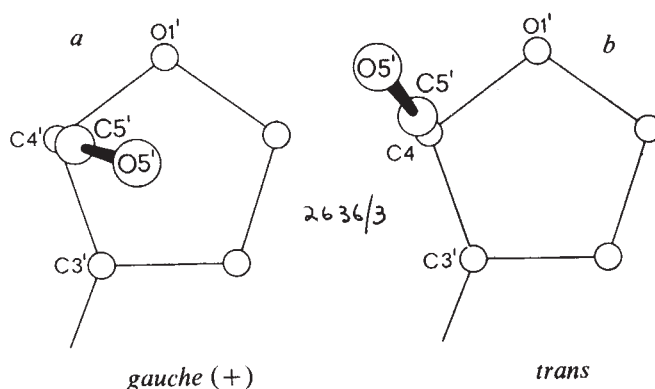
A number of very similar structures can be built along these lines and it is not obvious which is to be preferred. They all have certain features in common.

(1) The axes of the two straight parts of the DNA do not necessarily intersect exactly, but may be separated by only a small distance, d , typically about 1 Å or less. Note that d has a sign. (2) The angle between the two axes, α (projected, if necessary, on to a plane perpendicular to the line joining their points of nearest approach) is easily made more than 90° but approaches 100° with difficulty. The model shown in Fig. 1 has $\alpha = 98^\circ$. At the maximum angle (for any particular model) the backbone of one straight part starts to touch the backbone of the other chain of the other straight part. This contact, marked B in Fig. 1 has a (local) dyad axis. (3) If we define the kink point as the point where the local helix axes, on either side of the kink, intersect (or, if they do not intersect, then the midpoint of the shortest straight line between the axes) then the distance, D , from the kink point to the plane of the nearest base pair is appreciable and typically in the region of 7–8 Å.

To introduce our fourth point we must first consider the relationship between three successive straight portions; that is, two kinks in succession. We assume that every kink is exactly the same. The structure formed will depend on the precise number of base pairs between the kinks. (It should be remembered that the B form of DNA has an exact repeat after ten pairs.) For example, if there are ten (or a multiple of ten) base pairs between two kinks, the structure will bend round into, very roughly, three sides of a square. If there are five base pairs between kinks (or an odd multiple of five) then the structure will approximate to a zig-zag. In short the dihedral angle for three successive straight portions will depend on the exact number of base pairs between the two adjacent kinks.

We can now state our fourth point. (4) The kink imparts a small negative twist to the DNA. This is most easily grasped by imagining that the kink is made in two steps; first, the two base pairs to be unstacked are unstacked in the axial direction without kinking the backbone—this

Fig. 3 Diagrams of the deoxyribose ring showing the approximate conformation at the $C_4'-C_5'$ bond (a) in the normal straight B form of DNA, and (b) in the proposed kink; the two sugars affected are marked in Fig. 2.



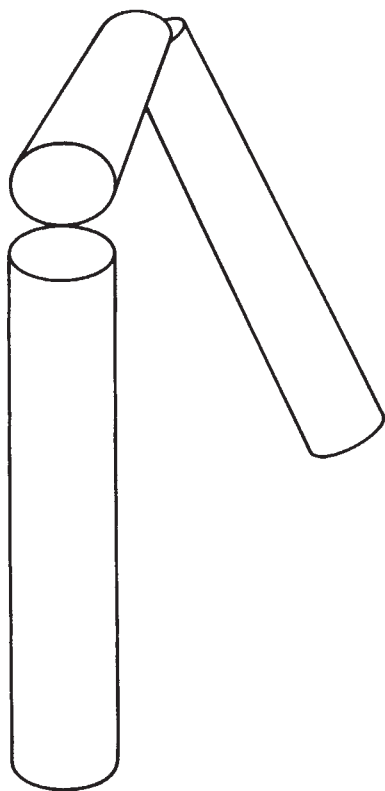


Fig. 4 Each cylinder represents a length of straight double helix. When there are ten base pairs (or an integer multiple of ten base pairs) in the middle stretch the kink has a left-handed configuration, as shown. The dihedral angle used in this paper is zero for the *cis* conformation. Its sign agrees with the usual convention that positive values less than 180° correspond to a right-handed configuration.

reduces the twist of 36° between these residues to about $10\text{--}20^\circ$ —and second, that this extended structure is then kinked. The result is that if successive kinks are made at intervals of $10n$ base pairs (where n is an integer) the DNA instead of folding back to form a 'circle' follows instead a left-handed helix, though naturally a kinked helix made of straight segments (see Fig. 4).

The exact dihedral angle associated with three successive straight stretches depends somewhat on the precise details of the kink but is typically about $(m \cdot 36^\circ - \theta)$ where m is the number of base pairs between the two kinks and θ , the dihedral correction angle, is not far from $15\text{--}20^\circ$. A very small rotational deformation of the straight portions could, however, alter this figure a little so the exact value in chromatin (if it does indeed consist of kinked helices) will probably be imposed by the histones.

(5) For any model there is a smallest number of base pairs between two adjacent kinks. For models of this family this number is usually three. In particular, the model illustrated in Fig. 1, for which $\alpha = 98^\circ$, can be built with three base pairs between two kinks but not with only two base pairs there. This is probably true for all models of this type for which α is greater than 90° . A model with three base pairs between two kinks exposes these base pairs rather effectively.

It is easy to see that six parameters are needed to describe the relationship between any two (equal) stretches of straight double helix. If these stretches are related by a dyad axis through the kink point then only four parameters are required. These can conveniently be taken to be the four used above: d , α , D and θ .

Another family of models can be made with the kink on the side of the major groove, but such structures have

a smaller angle of kink and seem rather awkward to build. We have not explored them further. A rather different type of kink, in which a base pair is undone, has been suggested by Gourévitch *et al.*¹⁴

The occurrence of kinks

The idea that the fold of DNA in chromatin was based on a unit of 10 base pairs was originally suggested to us by experimental evidence discovered by our colleague, Dr Markus Noll. Noll¹⁵ has shown that the digestion of native chromatin with the nuclease DNase I produces nicks in the DNA which tend to be spaced multiples of 10 bases apart. This suggests that the DNA is folded in a highly regular way and is probably mainly on the outside of the structure^{6,15}. More recent work by Noll and Kornberg (unpublished) using micrococcal nuclease points to a structural repeat at intervals of 20 base pairs. Thus a rather neat model for the most compact (wet) form of chromatin can be made in which the DNA is kinked through about $95\text{--}100^\circ$ every 20 base pairs, giving a shallow kinked helix having 10 straight stretches of DNA in each 100 Å repeat. The middle stretches of this repeat would be largely protected by the histones of the bead, the flanking stretches less so. Whether this very simple model is basically correct remains to be seen.

Obviously we should ask whether DNA is kinked in other situations. One interesting possibility is that when the *lac* repressor binds to the operator site on the DNA the double helix becomes kinked. It has been shown by Wang, Barkley and Bourgeois¹⁶ that this binding unwinds the helix by a small angle, either about 40° , or, more likely, about 90° (the value depending on the amount of unwinding assumed to be produced by the standard agent, ethidium bromide). As they point out, this is too small to allow the formation of a Gierer-type loop¹⁷. It is, however, just what one would expect from a small number of kinks since each kink of the kind we have described unwinds the double helix by about 15° to 25° . For example, an attractive zig-zag model can be imagined with four kinks, each spaced about five base pairs apart. This model places the two sequences related by a dyad, each of six consecutive base pairs, on either side of the first and last kinks (see Fig. 5). In this position, being near a kink, they are more exposed than they would be in a stretch of unkinked DNA.

In essence, kinking may be a way of partly exposing a small group of base pairs without too great an expenditure of energy. The exposed side of each of these base pairs is that normally in the major groove. The kink has the effect of displacing one of the phosphate-sugar backbones which normally make up the two sides of this groove. The specific pattern of hydrogen bonding sites in the major groove is thus made more accessible for a few base pairs on either side of a kink. A kink may therefore turn out to be a preferred configuration of DNA when it is interacting specifically with a protein.

Kinks may be suspected in all cases where double-stranded DNA has been shown to adopt a more compact

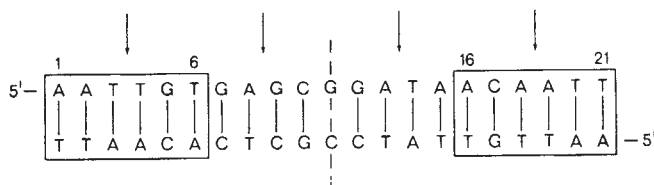


Fig. 5 The minimal base sequence of the *lac* operon, taken from Gilbert and Maxam¹⁸. The dotted line marks the pseudo-dyad in the base sequence. The two sets of consecutive base pairs, related by the dyad, are boxed. The arrows show one choice of positions where kinks might occur.

state than the normal double helix. Obvious examples are the folded chromosomes of *Escherichia coli*^{19,20} (and no doubt other prokaryotes), the folded DNA in viruses, the ψ phase of naked DNA discovered by Lerman²¹ and the shortened form of DNA in alcoholic solutions as described by Lang²².

One should also ask whether kinks occur spontaneously, as a result of thermal motion, in double-stranded DNA in solution. The frequency at which this occurs clearly depends on the free energy difference involved. If this were, say, about 4 kcalorie then there should be one kink in about 800 base pairs which could be appreciable. Such kinks would occur mainly between A-T pairs. If the free energy were as high as 6 kcalorie this would produce one kink in about every 22,000 base pairs, which would be more difficult to detect.

At the present we have no compelling evidence which shows that DNA in chromatin is kinked rather than bent nor that kinks exist in DNA in other contexts. Nevertheless our model seems to us sufficiently attractive to be worth presenting now for consideration by other workers in the field. Kinks, if they occur, have at least two possible advantages. It has always been a puzzle how to construct hierarchies of helices in a neat way, since bending an existing helix necessarily distorts its regular structure. This distortion becomes more acute as the basic helix is coiled at higher and higher levels. A kink allows such deformations to be local rather than diffuse and makes it easier to build hierarchical models which are neat stereochemically.

The other advantage is that, at a kink, several base pairs may be more easily available for specific interaction with a protein. If kinks in DNA exist they will surely prove to be important.

We thank our colleagues Drs R. D. Kornberg, M. Noll and J. O. Thomas for communicating their results to us before publication. We also thank them and our other colleagues for many useful discussions on chromatin structure.

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Isolation of histone genes from unfractionated sea urchin DNA by subculture cloning in *E. coli*

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Histone genes have been cloned selectively in Escherichia coli directly from unfractionated sea urchin DNA using labelled mRNA probe. The cloning method used is potentially applicable for isolating any genetic sequence for which a probe is available. The several eukaryotic gene fragments introduced into bacteria by this method collectively contain all or nearly all of the DNA sequences represented in histone mRNA.

THE ability to isolate eukaryotic DNA regions that code for specific gene products is vital to the understanding of gene organisation and function in higher organisms. Previously, separation of such regions from the bulk of eukaryotic DNA has been possible only when their nucleotide sequences have been highly repetitive or have had distinguishing physical properties, such as a skewed base-pair ratio¹⁻⁶. But now DNA molecules from prokaryotic and eukaryotic sources can be cloned in *Escherichia coli* as part of a bacterial plasmid replicon⁷⁻¹³ or bacteriophage genome¹⁴. So far the eukaryotic genes introduced into bacteria have been either purified species of DNA

separated from the bulk of the chromosome (such as *Xenopus laevis* rDNA)^{8,9}, or non-fractionated, by randomly inserted fragments of eukaryotic DNA (such as *Drosophila melanogaster* DNA^{12,14}). Such studies have shown that *X. laevis* rDNA is transcribed in *E. coli*⁸.

Since the histone-coding DNA sequences of sea urchins code for an identifiable set of mRNAs and proteins^{15,16}, they are of particular value for studies of macromolecular synthesis by eukaryotic DNA cloned in *E. coli*. Similarly, cloning of DNA segments carrying histone genes would provide an important tool for investigating the organisation of the reiterated histone DNA sequences on the eukaryotic chromosome^{17,18}. Histone genes have already been separated from bulk sea urchin DNA by physical methods⁶. We report here the isolation of histone genes directly from unfractionated sea urchin DNA by subculture cloning in *E. coli*. We used a radioactively labelled histone mRNA probe to identify bacterial clones containing histone genes. This procedure is applicable to the selection of any prokaryotic or eukaryotic DNA sequence for which a specific RNA probe is available.

Selection of histone DNA plasmids

A mixture of either unfractionated *Lytechinus pictus* or *Strongylocentrotus purpuratus* sea urchin DNA with pSC101 plasmid DNA (ratio 5:1) was cleaved by the *EcoRI* restriction endonuclease and ligated as described previously for *X. laevis*

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Table 1 Purification of sea urchin histone DNA-pSC101 chimaeras by subculture cloning

Sequential subculture steps	Detergent lysis hybridisation	Purification ratio	CCC-DNA hybridisation	Purification ratio
	c.p.m. hybridised per μg DNA c.p.m. hybridised per μg <i>L. pictus</i> DNA		c.p.m. hybridised per μg DNA c.p.m. hybridised per μg <i>L. pictus</i> DNA	
(1) <i>L. pictus</i> -pSC101 transformants	5.3/70.6	0.075	2.3/20.8	0.110
(2) Last positive row	58/67.4	0.87	7.0/20.8	0.337
(3) Grid row 1	86.6/54	1.60	13.7/20.8	0.659
(4) Grid column 4	52.2/54	0.97	10.4/20.8	0.5
(5) Grid intercept well	181/23.5	7.70	153/20.8	7.33
(6) Pool of intercept colonies	91/89.6	1.11	62/20.8	2.98
(7) Row A of pool	104/18.6	5.61	155/20.8	7.46
(8) Colony no. 8 (pLpl)	—	—	857/20.8	41.8

The presence of histone-DNA sequences among *E. coli* transformants was monitored by hybridisation of sea urchin histone ^3H -mRNA to plasmid chimaera DNA. For hybridisation, 50-ml overnight cultures of bacteria were subjected to detergent lysis²¹, the chromosomal DNA was pelleted by centrifugation at 20,000g for 30 min, and the supernatant was treated with Proteinase K (EM Laboratories, Elmsford, New York) (50 $\mu\text{g ml}^{-1}$) at 37 °C for 30 min. The lysate was extracted twice with equal volumes of phenol and once with chloroform. Cold ethanol (2.5 volumes) was added to the aqueous phase and a precipitate was allowed to form at -20 °C for at least 2 h. The voluminous precipitate was collected by centrifugation and resuspended in 1.0 ml of water. NaOH was added (final concentration 0.5 N) and the solution was allowed to stand at room temperature for at least 30 min to accomplish denaturation of plasmid DNA. The solution was neutralised and loaded on to nitrocellulose filters^{17,22}. Filters loaded with aliquots of DNA were hybridised with either sea urchin ^3H -histone mRNA at 52 °C or at 37 °C with complementary RNA (cRNA) transcribed³⁴ from pSC101 DNA by *E. coli* RNA polymerase (the enzyme was a gift of Dr K. Squires); both hybridisation reactions were carried out in 6×SSC containing 50% formamide for equal times ranging from 30 min to 18 h. Reaction vials also contained a 10- μg sea urchin DNA filter or a 1- μg pSC101 DNA filter where appropriate. Filters were batch-washed sequentially in 11 of 6×SSC, 2×SSC and 2×SSC containing pancreatic RNase (10 $\mu\text{g ml}^{-1}$), dried and counted in a liquid scintillation spectrometer¹⁷. In these conditions ^3H -histone mRNA does not hybridise to pSC101 DNA and pSC101 cRNA does not hybridise to sea urchin DNA. A filter containing 10 μg of *M. lysodeikticus* DNA was present in each vial as a blank and its counts (usually less than 50 c.p.m.) were subtracted from experimental values. Since the lysis procedure used recovers various proportions of plasmid DNA and *E. coli* chromosomal DNA, the amount of plasmid DNA on each filter was determined from the ratio of hybridisation of pSC101 ^3H -cRNA to pSC101 DNA and to the bacterial lysate DNA. From this value we calculated the c.p.m. of ^3H -mRNA hybridising per 1 μg of plasmid DNA shown in the Table. CCC-DNA plasmid was purified from subcultures at each step for comparison and was hybridised simultaneously with ^3H -labelled histone mRNA essentially as described above. The purification steps are described in the text. Bacteria from the initial culture of *L. pictus* DNA-pSC101 transformants (line 1) were diluted serially seven times (3:1 dilutions) in an 8×48 well grid made in sterile microtitre plates. The initial inoculum in the top well of each of the 48 columns was 10⁴ bacteria. The wells were then filled with L broth (0.25 ml) containing tetracycline (10 $\mu\text{g ml}^{-1}$) and incubated in a humidified atmosphere to prevent evaporation. After 24 h of incubation, several wells in the last two rows still had no growth suggesting that limit dilution had been reached. Aliquots taken serially from each of the 48 wells of each row were pooled. The eight resulting pools were cultured individually in 100 ml of L broth overnight and plasmid DNA was isolated as described above. The 48 wells in the last positive row (line 2) were arranged in a 6×8 grid and pools were made from each of the eight rows and six columns. DNA isolated from cultures of the first row (line 3) and the fourth column (line 4) hybridised to histone mRNA identifying the intercept well having bacteria containing histone DNA. DNA isolated from a culture of this single well (line 5) was markedly enriched for histone DNA sequences. Cells from this well were streaked on nutrient agar; 96 individual colonies were picked and placed in the wells of an 8×12 microtitre plate. After overnight incubation, aliquots from the wells of each row of this plate were pooled and cultured. DNA isolated from each culture was tested for the presence of histone DNA. One row (line 7) was positive and cells from the 12 wells in this row were individually cultured. DNA isolated from cultures of each well in the row was tested by RNA-DNA hybridisation. Cells from one well (line 8) gave a positive signal and was designated plasmid *L. pictus* 1 (pLpl).

rDNA^{8,9}. The DNA was then used to transform calcium chloride-treated cells of the restriction-deficient *E. coli* strain HB101. After the 42 °C heat-pulse DNA uptake step of the transformation procedure, bacteria were incubated in L broth and diluted into the same medium containing tetracycline (Tc, 25 $\mu\text{g ml}^{-1}$), as described previously^{19,20}. The cells were grown to stationary phase, and DNA isolated from the unfractionated heterogeneous population of Tc-resistant bacteria by detergent (Brij-58) lysis²¹ was extracted with a phenol-chloroform mixture, loaded on to nitrocellulose membrane filters^{17,22} and hybridised with the homologous radioactive *L. pictus* or *S. purpuratus* histone mRNA (Table 1). Covalently closed circular (CCC) DNA isolated²³ from the same bacterial culture was analysed by hybridisation for comparison. The specificity and authenticity of the histone mRNA used as a probe for the presence of histone DNA sequences has been documented previously^{16,24}.

We estimated that histone DNA sequences would be present initially in one of every 2,000–3,000 cells of the heterogeneous Tc-resistant transformed bacterial population. This estimate was derived from the observed frequency of chimaera formation when similar conditions were used for ligation of *Eco*RI-generated fragments of *X. laevis* rDNA to the pSC101 plasmid⁸ and the fraction (0.25%) of sea urchin DNA estimated to represent histone genes²⁵. Hybridisation of DNA isolated from the total transformed bacterial population with the tritium-labelled histone mRNA probe (Table 1, line 1) indicated that DNA sequences complementary to the RNA were detectable.

The specific bacterial cells containing histone DNA were cloned selectively from the heterogeneous population of Tc-resistant transformants by an adaptation of a method for

subculture cloning of bacteria²⁶. 10⁴ cells were placed in each of the 48 wells in the top rows of microtitre plates and diluted serially to a final concentration of approximately four cells per well. From the calculated frequency of histone DNA in the plasmid population, we estimated that by the fifth dilution, bacteria in only one of the 48 wells in the entire row would contain histone genes, which would be present in one of every 60 cells in that well. DNA isolated from a pooled cell population obtained from each row was tested for the presence of cloned histone DNA by hybridisation with ^3H -labelled sea urchin mRNA. We then used DNA-RNA hybridisation to identify the final dilution that contained a positive signal, to find the 'positive' well(s) in that row, and to purify further those specific bacteria that contained DNA complementary to the histone mRNA (see legend to Table 1).

Several experiments of this general design were carried out using eukaryotic DNA from *L. pictus* or *S. purpuratus*; Table 1 shows the results of a typical experiment. The amount of histone mRNA hybridising per μg of plasmid DNA increased at each stage of the isolation procedure, as transformants containing histone DNA were purified from the background of plasmids which lack histone DNA. The 48 wells of the row containing the last dilution that yielded a positive hybridisation signal (Table 1, line 2) were rearranged into a grid, and aliquots from the wells of each row and each column of this grid were pooled. We cultured the resulting pools of bacteria and then tested DNA isolated from each culture for the presence of nucleotide sequences which hybridised to histone mRNA. The resulting hybridisation pattern (Table 1, lines 3 and 4) facilitated identification of an intercept well (Table 1, line 5) containing bacteria which carried histone DNA plasmid chimaeras. Cells from this

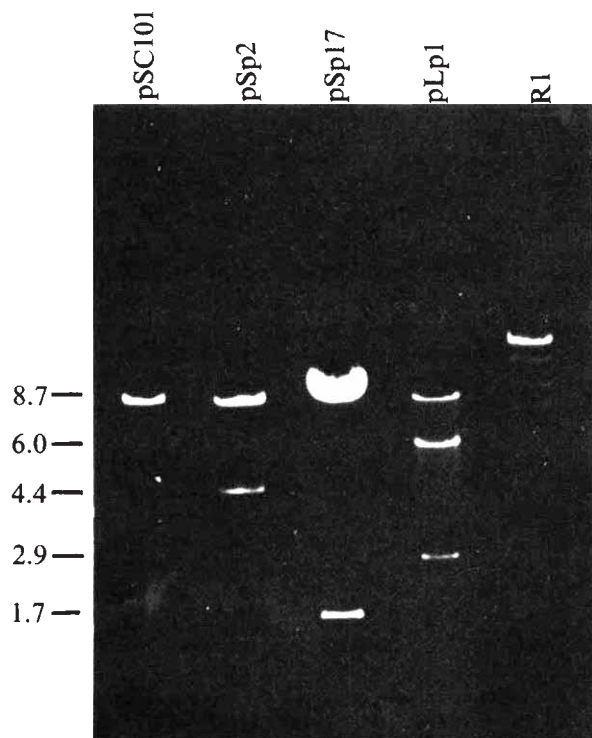


Fig. 1 Agarose gel electrophoresis of *EcoRI*-cleaved DNA of pLp1, pSp2, and pSp17 as well as pSC101 and R1 plasmids. The conditions used for *EcoRI* cleavage and for electrophoresis in 0.8% agarose gels have been described⁹. The sizes of fragments of plasmid chimaeras are expressed in kilobases (kb). Fragment size was calculated from mobility in the gel relative to the mobility of fragments of plasmid R1 DNA cleaved by the *EcoRI* endonuclease³³.

well were streaked on nutrient agar plates, and individual colonies were selected for further analysis by DNA-RNA hybridisation (see legend to Table 1).

This subculture cloning method, which can be carried out using either centrifuged detergent lysates of bacterial cells or purified plasmid CCC-DNA, enables rapid identification of those pools of bacteria containing plasmids that carry specific eukaryotic genes. It is potentially applicable for the isolation of any gene or gene combination which may be present in unknown quantities in a heterogeneous population of DNA, as long as a suitable probe is available. When cleared bacterial cell lysates are used, the procedure can be completed in 7–10 d when one cycle of lysis and hybridisation is performed daily.

In certain experiments, histone genes were first purified 60–70-fold by repeated centrifugation in caesium chloride-actinomycin D gradients (refs 6 and 26, and compare Fig. 4a) or alternatively, those plasmids containing randomly inserted fragments of sea urchin DNA were selected from a heterogeneous DNA population by repeated cycles of transformation and sucrose gradient centrifugation⁹ before cloning of the specific histone genes. When such initial purification was carried out, hybridisation results suggested that more than 1 in 1,000 plasmids contained histone genes. In these instances, we used a variation of our subculture cloning method; 100–150 bacteria obtained from cultures yielding a positive signal were cultured to stationary phase in each of ten flasks. Aliquots were set aside, and DNA isolated from the remaining bacteria in each flask was assayed by DNA-RNA hybridisation for the presence of histone genes. The cells from flasks which had proved positive were subcultured by regrowth in ten additional flasks (10–15 cells per flask) and tested once more for DNA which hybridised with histone mRNA. Bacteria from flasks showing a positive 'signal' were streaked on agar plates; randomly selected colonies from each plate were cultured separately in

liquid media, and the clones containing histone genes were identified by DNA-RNA hybridisation.

These various methods permitted the isolation of bacterial clones which contained *L. pictus* or *S. purpuratus* DNA complementary to histone mRNA. One clone containing *L. pictus* DNA (Table 1, line 8) and two containing *S. purpuratus* DNA were selected for further study.

Characterisation of plasmid chimaeras

Covalently closed circular plasmid DNA isolated from these three clones was cleaved by the *EcoRI* restriction endonuclease, and examined by agarose gel electrophoresis (Fig. 1). Each

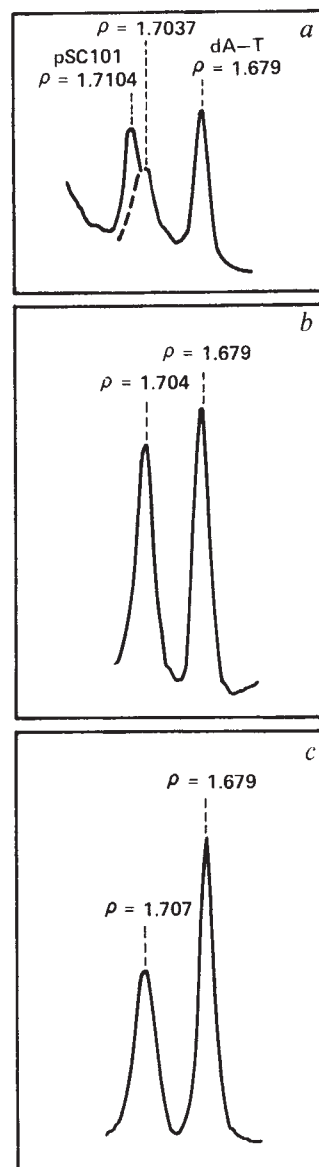


Fig. 2 CsCl gradient centrifugation analysis of plasmid chimaera DNA. *a*, *EcoRI* digest of pLp1 DNA. Separate peaks banding at $\rho = 1.710 \text{ g cm}^{-3}$ (pSC101 fragment) and $\rho = 1.703 \text{ g cm}^{-3}$ (eukaryotic DNA fragment) are seen. The eukaryotic DNA fragments of the pSp2 (4.4 kb) (*b*) and pSp17 (1.7 kb) (*c*) plasmids were separated from the pSC101 DNA fragments by agarose gel electrophoresis⁹ of *EcoRI*-cleaved plasmid DNA before centrifugation. Gel slices containing ethidium bromide-stained DNA fragments were excised and crushed, and the DNA was eluted at 37 °C for 2 h in 10 mM Tris-HCl 1 mM EDTA, pH 8.0. Recovery was 60%. The buoyant densities of the eukaryotic DNA fragments and of the dA-T marker ($\rho = 1.679 \text{ g cm}^{-3}$) are shown. The buoyant density we observed for unfractionated *L. pictus* and *S. purpuratus* DNA preparations (not shown) was $\rho = 1.697 \text{ g cm}^{-3}$. Conditions for centrifugation have been described²³.

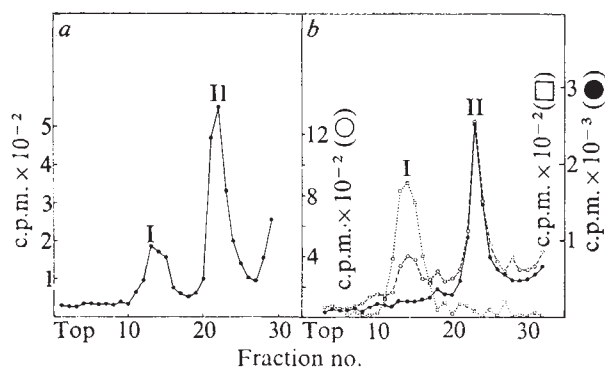


Fig. 3 Sucrose density gradient sedimentation analysis of *Eco*RI-cleaved pSp17 DNA. ^3H -thymidine-labelled¹⁹ or unlabelled CCC pSp17 DNA was incubated with *Eco*RI restriction endonuclease in the conditions described in the legend for Fig. 1. After heat inactivation of the enzyme at 65 °C for 5 min, the lysates were layered directly over 5–20% sucrose gradients in 1 N NaCl, 0.01 M Tris, 1 mM EDTA and centrifuged at 50,000 r.p.m. for 3.5 h at 22 °C in a Beckman SW 50.1 rotor. Six drop fractions were collected by piercing the tube at the bottom and pumping the contents through an ISCO fractionator. *a*, After centrifugation, *Eco*RI digested ^3H -thymidine-labelled pSp17 DNA was collected directly on to Whatman glass fibre filters in scintillation vials, dried and counted in a Beckman liquid scintillation spectrometer. Two peaks of radioactivity (I and II) were found. *b*, 100 μg of pSp17 DNA was digested with *Eco*RI, divided, and centrifuged on two sucrose gradients with the gradient in (*a*). The collected fractions were divided and plated on each of two 13-mm nitrocellulose membrane filters as described in the legend to Table 1. The dried filters were numbered and cut in half. Each set of split filters was hybridised in 1 ml of 6 $\times$ SSC-50% formamide containing various radiolabelled RNAs: $\circ$, cRNA synthesised by *E. coli* RNA polymerase with pSp17 DNA as template; $\bullet$, cRNA with pSC101 DNA as template; $\square$, sea urchin histone mRNA. The hybridisation peaks I and II correspond to the radioactively labelled DNA peaks I and II in (*a*).

plasmid contained at least one DNA fragment in addition to the 8.7 kilobase (kb) DNA species characteristic of the pSC101 plasmid. The *L. pictus* plasmid chimaera (pLp1) contained two additional DNA fragments with calculated lengths of 6.0 kb and 2.4 kb. Two distinct types of clones containing *S. purpuratus* DNA were observed; one (pSp2) contained a DNA fragment 4.4 kb long (pSp2), whereas the other contained a 1.7 kb DNA fragment (pSp17).

Caesium chloride centrifugation analysis of *Eco*RI-treated pLp1, pSp2, and pSp17 DNA (Fig. 2) indicated that the additional DNA fragments present in all three plasmids have higher densities than the bulk of sea urchin DNA. The observed values are in close agreement with earlier buoyant density estimates for histone genes of sea urchins^{6,17,18}.

Sucrose gradient centrifugation of *Eco*RI-cleaved, radioactively labelled pSp17 DNA (Fig. 3*a*), demonstrates two endonuclease-generated plasmid fragments (peak I and peak II) with sedimentation properties consistent with the lengths calculated from their mobilities in agarose gels (1.7 kb and 8.7 kb, respectively). Aliquots taken from fractions of a similar sucrose gradient containing unlabelled *Eco*RI cleaved pSp17 DNA were plated on nitrocellulose membranes, and were hybridised in solutions containing either: (1) *S. purpuratus* histone mRNA, (2) complementary RNA (cRNA) synthesised *in vitro* from pSp17 plasmid DNA using the *E. coli* RNA polymerase, or (3) similarly prepared cRNA transcribed from pSC101 plasmid DNA (Fig. 3*b*). Hybridisation with sea urchin histone mRNA was localised to only peak I (the 1.7 kb fragment) of the plasmid chimaera. Both DNA fragments of the pSp17 plasmid contain nucleotide sequences homologous with pSp17cRNA and as expected, the pSC101 cRNA synthesised *in vitro* hybridised with only the faster sedimenting peak II DNA fragment.

Nine or ten unique RNA species, which code for the several

sea urchin histones, are contained in the *S. purpuratus* and *L. pictus* histone mRNA probes used in these experiments^{16,24}. To determine whether the two cloned species of eukaryotic DNA were complementary to all or to only some of the RNA sequences in the *S. purpuratus* probe, we hybridised this mRNA with an excess of DNA of each *S. purpuratus* plasmid chimaera. In similar conditions of DNA excess we found that total *S. purpuratus* DNA can anneal with 65–70% of the input RNA (Table 2*a*). In parallel reactions, pSp2 DNA hybridised with 41–44% of the input RNA sequences while pSp17 annealed to 35%. When filters containing DNA from both plasmid chimaeras were included in the same hybridisation mixture, 83% of the input RNA annealed (Table 2*a*). Thus DNA sequences contained in the 1.7 kb and 4.4 kb cloned sea urchin DNA fragments seem to be mostly non-homologous, and together these two cloned DNA fragments probably code for most if not all of the histone mRNA sequences present in *S. purpuratus*. This result also suggests that DNA coding for the several different histones of *S. purpuratus* is clustered within the confines of the two eukaryotic DNA fragments we have cloned and implies that at least some of the repetitive DNA sequences coding for the various *S. purpuratus* histones are intermingled with each other. In addition, the high percentage of histone mRNA sequences homologous with the two cloned eukaryotic DNA fragments in this experiment precludes the possibility that our hybridisation results are attributable to the small fraction of non-histone mRNA (~10%) which routinely contaminates histone mRNA preparations^{16,24}.

Histone genes in *S. purpuratus* are repeated some 400–500 times²⁵ and are chromosomally linked into one or a few clusters^{17,18}. Since the two *S. purpuratus* chimaeric plasmids

Table 2 Hybridisation data

(*a*) Hybridisation of sea urchin histone mRNA to plasmid chimaera DNA

DNA (μg)	% Hybridised
<i>S. purpuratus</i> DNA	
10	32
20	50
30	72
40	65
pSp2 DNA	
10	44
20	41
pSp17 DNA	
10	36
20	35
pSp2 10 } pSp17 10 }	48 } 35 } 83

(*b*) Hybridisation of ^3H -RNA synthesised by *E. coli* minicells containing plasmid chimaeras

DNA on filter	Hybridisation (c.p.m.) of RNA made by:		
	pSC101	pSp17	pSp2
<i>M. lysodeikticus</i>	44	32	37
<i>S. purpuratus</i>	33	637	957
pSC101	8425	7225	2631

a, 850 c.p.m. of trichloroacetic acid precipitable *S. purpuratus* histone mRNA was hybridised with nitrocellulose filters containing the amount of DNA listed. Each reaction was performed in 1 ml of 2 $\times$ SSC-0.2% phenol at 67 °C for 14 h. The filters were washed as described in Table 1. The c.p.m. hybridised to the individual filters have been corrected for the number of counts hybridising to a filter containing 10 μg of *M. lysodeikticus* DNA (less than 40 c.p.m. in each case).

b, Excess RNA (75 $\times$ 10³ or greater c.p.m.) was placed in 0.4 ml of 6 $\times$ SSC-50% formamide containing a 10- μg *S. purpuratus* filter, a 10- μg *M. lysodeikticus* filter, and a 1- μg pSC101 filter, and incubated at 52 °C overnight. The filters were washed and treated with RNase as described in the legend to Table 1.

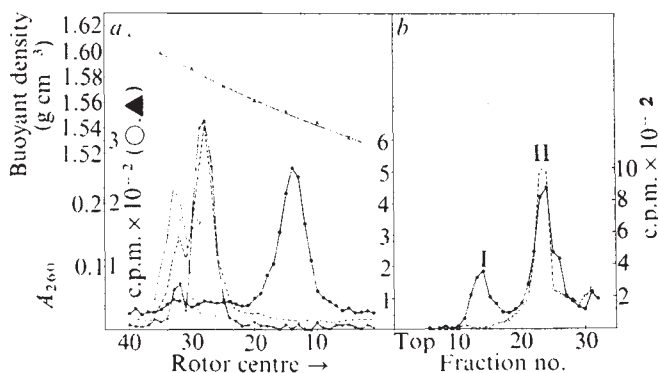


Fig. 4 Hybridisation of RNA transcribed by minicells containing histone DNA plasmid chimaeras. *a*, Hybridisation of minicell transcripts to sea urchin DNA analysed on caesium chloride-actinomycin D buoyant density sedimentation gradients. 1 mg of sea urchin DNA was complexed with an equal weight of actinomycin D (a gift of Merck, Sharpe and Dohme) and centrifuged to equilibrium in CsCl^{6,32}. The gradient was fractionated and aliquots of each fraction plated on nitrocellulose filters as described in the legend to Table 1. Multiple sets of filters were made and each hybridised with different radiolabelled RNA preparations. The filters were then batch-washed, treated with RNase A and counted as described in the legend to Table 1. —, A_{260} of main band sea urchin DNA; ●, hybridisation of tritiated rRNA prepared from chick muscle; ○, hybridisation of sea urchin ³H-mRNA; ▲, hybridisation of ³H-uridine minicell RNA from pSp17 plasmid. *b*, Hybridisation of minicell RNA with *Eco*RI digested pSp17 DNA fractionated on a sucrose density gradient. Fractions taken as aliquots from the sucrose gradient shown in Fig. 3b were hybridised with 1.2×10^4 c.p.m. of ³H-uridine labelled RNA made by pSp17-containing minicells (●) or with 1.6×10^4 c.p.m. of RNA transcripts produced by pSC101-containing minicells (○). Filters were treated as described above.

collectively contain DNA sequences complementary to all or almost all the histone mRNA sequences, they must contain genes for several different histone proteins. Evidence for a single histone DNA locus obtained from the organisation of histone DNA in *D. melanogaster*^{27,28} suggests by analogy that the two *S. purpuratus* fragments are sections of a repeat unit 6 kb long. A unit of this size repeated 470 times²⁵ would represent approximately 0.3% of the sea urchin genome²⁹ and corresponds well with previous estimates^{24,25}. Our method of selection for histone DNA fragments would not detect histone gene spacer sequences cleaved from coding regions by *Eco*RI restriction endonuclease, and a complete analysis of histone DNA structure may require examination of longer histone DNA fragments.

Transcription in *E. coli* minicells

Plasmid chimaeras containing *X. laevis* rDNA can segregate into *E. coli* minicells⁸ when these cells are budded off from the parent *E. coli*³⁰. RNA complementary to the eukaryotic gene sequence is synthesised in such minicells⁸. The synthesis of RNA products by eukaryotic genes introduced into bacterial minicells provides an additional parameter for characterising the DNA fragments cloned in the studies reported here. The minicell-producing *E. coli* strain P678-54 was transformed³⁰ to Tc resistance with pSp2 and pSp17 plasmid DNA. CCC-DNA isolated from purified minicells and treated with the *Eco*RI endonuclease contains agarose gel DNA fragments identical in mobility to those seen with the parent plasmid chimaeras. Minicells containing the plasmids were incubated with ³H-uridine³¹, and RNA purified from these cells was hybridised with total sea urchin DNA immobilised on nitrocellulose membranes. The results (Table 2b) show that RNA able to anneal with the eukaryotic DNA is synthesised in *E. coli* minicells carrying either of the recombinant plasmids, but not by minicells carrying the pSC101 plasmid alone.

Tritiated RNA isolated from minicells containing the pSp17 plasmid was hybridised also to total sea urchin DNA fractionated by centrifugation in actinomycin D-caesium chloride

gradients^{6,32}. Our results demonstrate that the RNA transcribed from the sea urchin gene sequences in minicells hybridises entirely with the satellite bands previously identified as histone DNA (Fig. 4a). No complementarity of the RNA made in *E. coli* minicells with either main band sequences or ribosomal DNA sequences was observed. An additional hybridisation experiment (Fig. 4b) demonstrated that RNA sequences made in *E. coli* minicells containing the pSp17 plasmid chimaera are transcribed from the eukaryotic DNA fragment of the plasmid as well as from the pSC101 segment (a similar result was obtained with the pSp2 plasmid). The amount of hybridisation of minicell RNA with each of the two component fragments of the plasmid chimaera seemed to be roughly proportional to the fragment size.

The subculture cloning procedure described here has enabled identification of individual transformed *E. coli* cells containing histone genes from among a total of 2×10^3 – 3×10^3 transformants. Other experiments in our laboratories indicate that the method can identify specific gene sequences present at a frequency as low as 10^{-6} . The procedure does not require previous purification of histone DNA sequences or expression of the genes in the bacterium. The technique is potentially applicable for the isolation in *E. coli* of any eukaryotic or prokaryotic DNA fragment for which a specific DNA or RNA probe is available. Moreover, the isolation and purification of groups of genes that function concurrently at different times during the life cycle of complex higher organisms now seems feasible.

Electron microscope analysis of heteroduplex molecules formed between plasmids pSp2 and pSp17 demonstrates no detectable homology of their eukaryotic DNA sequences. In addition, sequences of the eukaryotic fragments from these two plasmids are present within a DNA fragment 6 kb long which is generated by limit digestion of *S. purpuratus* DNA with the restriction endonuclease *Hind* III. Thus the two *Eco*RI derived fragments are in close proximity in the sea urchin chromosomal DNA and the sequences coding for most if not all of the several histone proteins are therefore closely linked within 6,000 base pairs. The details of these experiments will be presented elsewhere.

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letters to nature

Taylor columns in a shear flow and Jupiter's Great Red Spot

THE Great Red Spot (GRS) on Jupiter is not located in a uniform flow but occupies a position at which the zonal wind varies strongly with latitude¹. The conditions in which Taylor columns form in these circumstances are critically dependent (J. W. Cottrell, unpublished; Woods Hole Oceanographic Institution G.F.D. Summer Programme. Student notes, 1971.) on the sign of the relative vorticity associated with the shear. On Jupiter the GRS is situated at a latitude where conditions are particularly favourable for the formation of a Taylor column, according to this model. Complementary to this theoretical approach we report here some preliminary results of the first experimental investigation into the effect of shear on the formation of a Taylor column.

The suggestion² that the GRS could be evidence of a naturally occurring Taylor column—a phenomenon observed in the laboratory when slow, steady flows pass a solid obstacle in a rapidly rotating fluid—has received much attention. Ideas that Jovian atmospheric motions are coupled to topographic features have stimulated several experimental investigations (see, for example, ref. 3) and these have been complemented by related observational data⁴ from the terrestrial oceans which confirm the importance of bottom topography in determining certain features of oceanic dynamics.

In the case of Jupiter, controversy has arisen over doubts concerning the existence of a solid surface on the planet (the 'topographic feature' is understood to be a mountain or, more probably, an impact crater formed on this surface). The conditions in which 'Taylor columns' form, however, are sufficiently general not to preclude the possibility that they could occur above any large scale irregularity⁵, such as a 'floating raft'—an alternative model for GRS proposed on other grounds⁶.

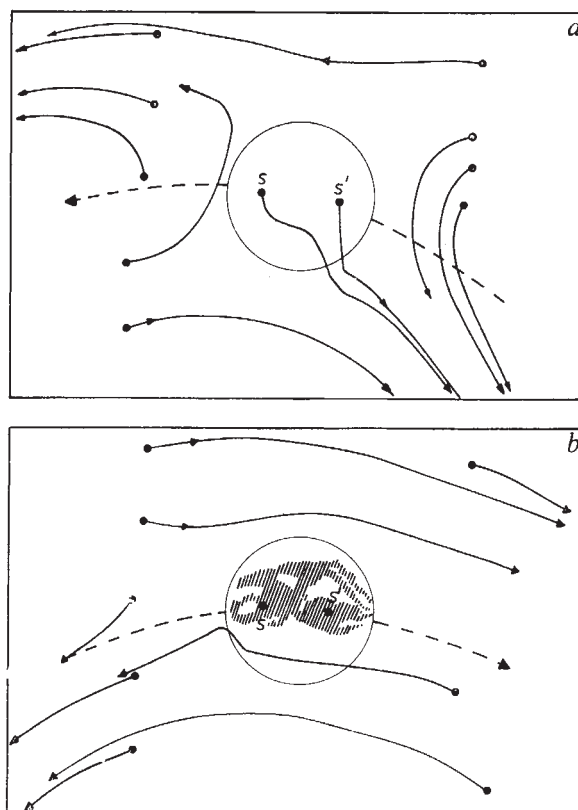
The possibility that such a mass of hydrogen-rich solid could float in a fluid layer of hydrogen and helium has received support from laboratory experiments on the phase behaviour of light gas mixtures⁷. Those results indicate that the development of a so-called 'Cartesian diver' model, and the accompanying formation of a Taylor column, are encouragingly similar to several established characteristics⁸ of the GRS (change in size, and longitudinal oscillations, for example). Preliminary reports from the Pioneer 10 and 11 missions supposedly add support to the 'long lived storm' hypothesis⁹ in spite of earlier criticism¹ of this model. Observations⁸ of the anticyclonic circulation of the GRS, on which the terrestrial hurricane analogue is based, refer to the motions in the neighbourhood of the spot and not to motions in its interior (see ref. 1). Indeed, it is noticeable that though the most recent high resolution photographic data¹⁰ reveal some structural details of the GRS, they do not indicate any of the Coriolis effects, such as spiralling motions, usually associated with plan views of terrestrial hurricanes, nor is the shape of the GRS characteristic of such phenomena. Additionally, the 'storm' hypothesis cannot explain the relatively in

significant latitudinal motion of the GRS and its longevity (it has existed for at least 300 yr).

As yet, there does not seem to be sufficient evidence to justify abandoning the Taylor column hypothesis, in some form, in favour of other proposed models (of which the 'long lived storm' seems to be the most plausible); when shear in the zonal flow is taken into consideration the Taylor column hypothesis becomes even more attractive.

Cottrell (unpublished) used the linearised, inviscid equations of motion to consider shear flow past an obstacle, the height of which could be varied. If the sense of ζ_∞ (the upstream relative vorticity associated with the shear) is positive with respect to the basic rotation then, above a critical obstacle height, given by $h = 2\epsilon\zeta_\infty$, all fluid contacting the imaginary vertical cylinder circumscribing the obstacle is returned, and a

Fig. 1 Plan views of dye-line paths at a height of 3 cm above the top of a cylindrical obstacle of height 1 cm and radius 2.5 cm for: *a*, positive and *b*, negative shear. The sense of the basic rotation is anticlockwise. Dye has been released simultaneously ahead of and behind the obstacle and from two sources, S and S', within the imaginary vertical cylinder circumscribing the obstacle. The hatched area in *b* represents the accumulated dye from S and S'.



region of closed streamlines forms above the obstacle. Here, ε is the Rossby number of the flow, defined as $\varepsilon = \zeta_\infty/2\Omega$, where Ω is the basic rotation rate. This is equivalent to the critical height derived² for uniform flow. In the case of negative ζ_∞ , however, all streamlines are closed for any finite value of h , and within the circumscribing cylinder the streamlines are elliptical and aligned with the direction of flow. In this circumstance, the area of the region of closed streamlines above the obstacle (the Taylor column) is directly proportional to the height of the obstacle.

The precise definition of a Taylor column has caused a good deal of confusion¹¹: as described mathematically by the Taylor-Proudman theorem^{12,13} they do not exist in nature, nor indeed in the laboratory and much effort has gone into determining the detailed structure of the real flow above a slowly moving obstacle in a rotating fluid. Though to the first order the fluid is trapped in the imaginary vertical cylinder circumscribing the obstacle, it is not stagnant, and secondary flows, associated with transport of fluid through the column 'walls', do exist. Here, we find it unnecessary to formulate a numerical criterion for the formation of a Taylor column based on the magnitudes of secondary flows. Instead, we are concerned in a more qualitative way with an obstacle's steering effect¹¹ on the flow at heights well above the obstacle, and the degree of trapping of fluid above it.

Our apparatus, described in detail elsewhere¹⁴, consists of a rotating cylindrical tank of fluid in which a basic azimuthal flow inversely dependent on the radius of the tank is generated by means of a source-sink arrangement⁸. A cylindrical obstacle (of a dimension typically 1/15 of the tank diameter) is rotated azimuthally through the basic flow at a velocity such that, relative to the obstacle, the flow is forward near the centre of the tank and reversed near the rim. Between these extreme positions there is a monotonic variation in the relative azimuthal velocity. The obstacle is thus situated in a horizontal shear flow, the sense of which can easily be changed by reversing the source-sink pumping arrangement and the sense of rotation of the obstacle. An array of vertical dye-generating electrodes¹⁵ is attached to the obstacle in order to visualise the flow, and a camera fixed to the rotating turntable records the plan view of the flow pattern.

Preliminary qualitative results agree encouragingly with predicted flow patterns (J. W. Cottrell, unpublished) and noticeable differences between situations in which the relative vorticity associated with the horizontal shear is positive or negative with respect to the vorticity of the basic rotation are confirmed (see Fig. 1). Furthermore, variations in obstacle height have a significant effect on the trapping of fluid above the obstacle. Definite Taylor column effects were observed with all cases of negative vorticity flows, though in situations where the relative vorticity was positive only obstacles above a certain size gave rise to flows like those associated with Taylor columns. This result supports qualitatively the theoretical prediction (J. W. Cottrell, unpublished) that in shear flows of negative vorticity Taylor columns can be expected to form in the presence of all obstacles of finite heights, whereas for uniform flow or for positive shear flow a minimum obstacle height is required for Taylor column formation.

As already stated, the GRS occupies one of the few regions of high negative vorticity on Jupiter, so conditions for the formation of a Taylor column at this location are particularly favourable. It would seem that the original Taylor column hypothesis², considered in conjunction with the possibility that a hydrogen-rich solid can exist in equilibrium with a hydrogen-helium liquid phase⁷ and with the results concerning the effects of horizontal shear, leads to a plausible model for the GRS, in which many of the prominent features (occurrence, shape, uniqueness, short period longitudinal oscillation) are explained. Detailed quantitative experiments on the effect of shear over a wide parameter range are being conducted at present. The possible modifying effect (J. W. Cottrell, unpublished) of the variation with latitude of the local vertical component of the rotation rate (the so-

called β effect) are also to be considered in this series of experiments. It is hoped that these quantitative results will enable the parameter range under which Taylor columns form in a shear flow to be established more precisely in order that the extent to which topographic coupling with the Jovian atmosphere, as an explanation of the GRS, can be gauged.

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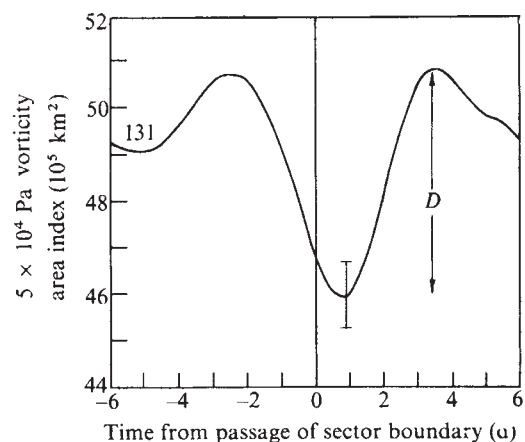
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Seasonal variation and magnitude of the solar sector structure-atmospheric vorticity effect

A RELATIONSHIP between the solar sector structure, as swept past the Earth by the solar wind, and terrestrial atmospheric vorticity has been reported by Wilcox *et al.*¹. The solar sector structure is a large scale property of the Sun that is seen most readily in its magnetic field. As observed by spacecraft magnetometers near the Earth, the extended solar magnetic field typically consists of four sectors within a 27-d synodic solar rotation period. Within each sector the magnetic field is predominantly either towards or away from the Sun. The sectors are separated by thin current sheets that reverse the direction of the field and constitute sector boundaries. Here we report evidence of a seasonal variation in this effect.

In the interval from about two days before a boundary is swept past the Earth by the solar wind to about four days after,

Fig. 1 Average response of the 5×10^4 Pa vorticity area index to the solar magnetic sector structure during the winter months November to March of the years 1963 to 1973. Sector boundaries were carried past the Earth by the solar wind on day zero. The probable error of the mean is indicated with an error bar, and the depth D of the minimum with respect to the average of the two adjacent maxima is indicated.



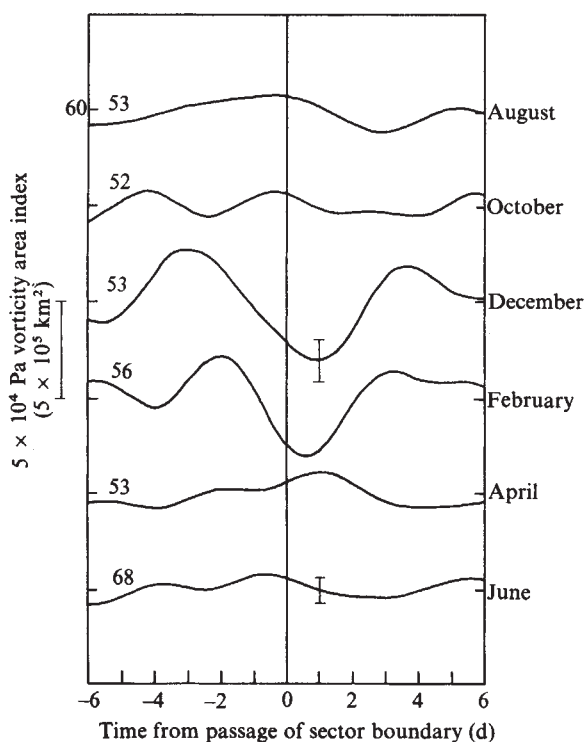


Fig. 2 Seasonal variation of the solar sector-vorticity area index effect shown in Fig. 1. The analysis leading to each curve is the same as the analysis leading to Fig. 1, except that for the curve labelled August, 60 boundary passages in the interval from July 16 to September 15 have been included, with analogous analyses for the other curves. The average value of the index for each curve is indicated with tick marks. Error bars are shown corresponding to the largest and the smallest s.e.m. for one day after the boundary passage. The ordinate range shown for $5 \times 10^5 \text{ km}^2$ applies to all six curves. The curves have been displaced in the vertical direction for clarity.

the vorticity area index² declined by about 10%, reaching a minimum about one day after the boundary. (The vorticity area index is a measure of the size and prominence of all the low pressure troughs in the region of the Northern Hemisphere north of 20°N .) Several properties of the Sun (and, by extension, of the solar wind) vary in an organised manner throughout each sector. The vorticity area index responds to these large scale variations in the sector structure. The precise time at which a boundary is swept past the Earth provides a convenient and well-defined timing marker. A description of the solar sector structure, its extension past the Earth by the solar wind, and the resulting effect on the terrestrial vorticity area index is given elsewhere³.

The previous analysis¹ has been repeated with the time extended to the interval 1963–73 and the number of cases increased from 54 to 131. We have used the $5 \times 10^4 \text{ Pa}$ (500 mbar) vorticity area index of Roberts and Olson² with a discriminator setting of $20 \times 10^{-5} \text{ s}^{-1}$. The results are very similar³ at $3 \times 10^4 \text{ Pa}$ and at $5 \times 10^4 \text{ Pa}$; here we use $5 \times 10^4 \text{ Pa}$ because the observations are more homogeneous. The index has been filtered to remove variations corresponding to periods greater than 13 d and less than 3 d, to remove the effects of seasonal variations and of short term fluctuations. The results (Fig. 1) show the average variation of the vorticity area index as the solar sector structure is carried past the Earth by the solar wind. The results are very similar to those reported previously¹ although the number of sector boundaries analysed has increased from 54 to 131.

The magnitude of the effect is substantial. The depth D of the minimum as defined in Fig. 1 is about $4.6 \times 10^5 \text{ km}^2$ which

is approximately 10% of the average value of the index. The area of the low pressure troughs where the vorticity is greater than $20 \times 10^{-5} \text{ s}^{-1}$ in the winter Northern Hemisphere decreases on the average by about 10% with a minimum one day after a sector boundary passage.

Alternatively, we may discuss the amplitude of the effect in terms of the standard deviation of the data as compared with the depth D of the minimum. The standard deviation is $12.3 \times 10^5 \text{ km}^2$, so that the depth D ($4.6 \times 10^5 \text{ km}^2$) of the solar sector-related effect is about one-third of the deviations.

To investigate the seasonal variation of the effect, the analysis leading to Fig. 1 has been repeated using two-month intervals (Fig. 2). The top curve labelled August includes the interval from July 16 to September 15, with analogous intervals for the other curves. The effect shown in Fig. 1 is clearly seen in the winter curves of Fig. 2 labelled December and February, but is not seen in the curves corresponding to the other seasons. A similar curve (not shown) computed for January was very similar to the curves for December and for February, suggesting that the effect is of approximately constant average amplitude through the winter. The fact that this effect is prominent only in winter may be related to the suggestion by King⁴ that the Earth's magnetic field may influence, through some unknown mechanism, the behaviour of the lower atmosphere at high latitudes in winter.

In Fig. 2 the curves labelled December and February represent separate analyses of 53 and 56 sector boundary passages. The similarity of each of these separate curves to the result shown in Fig. 1 is further evidence for the reality of the effect.

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Distribution function for random distribution of spheres

THE statistical problems associated with a distribution of spheres in space have received considerable attention^{1–3}, particularly in the limiting cases of very small volume concentrations and very large ones (close to maximum packing). The intermediate range of finite concentration is not as well covered, and this range is considered here.

The difficulties connected with the definition of a random distribution of spheres have been considered elsewhere^{1,2} and here the following construction will be used. Let ϕ be a given volume concentration and N the number of spheres per unit volume; the radius a of each sphere is given by $\phi = (4/3)\pi Na^3$. Using the convention that the position of a sphere is specified by the position of its centre, one chooses a point P_1 at random in a unit volume and places a sphere there. Then a point P_2 is chosen at random and the distance P_1P_2 is computed. If this distance is greater than $2a$, a sphere is placed there; otherwise the point is rejected. Then a point P_3 is chosen and the same test applied to P_1P_2 and P_2P_3 . The procedure stops when N spheres are embedded in the unit volume.

Buevich⁵ has proposed a distribution function for a random distribution of spheres:

$$P(i;V) = C_i^n (1-s)^{n-i} s^i, \quad i \leq n \quad (1)$$

where $P(i;V)$ is the probability that the set of measure V contains i particles; n is the maximum number of particles which may be embedded in V ; $s = \phi/\phi_m$, where ϕ_m is the volume fraction corresponding to the maximum packing ($\phi_m = 0.907$ in two dimensions and $\phi_m = 0.74$ in three dimensions) and C_n^m is the binomial coefficient.

Equation (1) follows from the assumptions that n is proportional to V , and a 'chaos' hypothesis in the form

$$P(m; U+V) = \sum_{i=0}^m P(i;U) P(m-i;V) \\ \text{for } U \times V = \phi.$$

These assumptions are simpler and more natural than those used in ref. 5, but a difficulty remains concerning the first assumption. There exist sets of an arbitrary measure in which no sphere can be embedded and so the first assumption implies restrictions on the type of sets considered. What these restrictions should be is not clear.

I have been interested (in the context of the theory of suspensions) in the distribution of nearest neighbours in a random distribution and I have conducted numerical experiments to check equation (1) in this context. The nearest neighbour distribution (three-dimensional case) associated with equation (1) is

$$f(R) = -32\pi n R^2 \ln(1-s) \cdot (1-s)^{(2/3)} \pi n (R^3 - a^3) \quad (2)$$

where $n = \phi_m / ((4/3)\pi a^3)$. The Poisson distribution function and its related nearest neighbour distribution function are contained in equations (1) and (2) as the limit $\phi = 0$ (when the spheres become points).

A distribution of points was generated as described above and the deviations of the experimental results for this distribution from the values predicted by the Poisson distribution function were obtained and used as references for the work on finite spheres. Similarly the deviations of the experimental and theoretical nearest neighbour distributions were computed.

Distributions of finite spheres were then generated and expressions (1) and (2) were checked against the numerically calculated functions. When computing the distribution functions for the experiments, the spheres close to the walls of the cube were ignored so as to avoid possible boundary influences. The experiments were carried out in both two and three dimensions, the maximum concentrations being respectively 0.39 and 0.22 ($N = 490$ and 725). Attempts to generate greater concentrations were stopped when 50 consecutive points had been rejected.

The deviations between the experimental values and those predicted by equations (1) and (2) were, over the whole range of concentration obtained, of the same order as the reference deviations. These results can be taken as experimental verification of equations (1) and (2).

For a mixture of spheres of two radii a_1 and a_2 the form of equation (1) remains unaltered except for n becoming

$$n = (3/4\pi) \phi_m (d_1 a_1^3 + d_2 a_2^3)^{-1}$$

where the d_i are relative numerical concentrations, so that if n_i is the number concentration of the i particles, $n = n_1 + n_2$, then $d_i = n_i/n$.

Equation (2) becomes

$$f(R) = \sum_{i,j} d_i d_j f(R; a_i, a_j)$$

where

$$f(R; b, c) = -32\pi n R^2 \ln(1-s) \cdot (1-s)^{(4/3)} \pi n (8R^3 - (b+c)^3)$$

For a mixture of particles the value of the maximum volume concentration ϕ_m depends, in general, on the ratio a_2/a_1 and d .

But the results obtained in the numerical experiments with the ratio a_2/a_1 between 1 and 2 and for d between 0.1 and 0.9 with a constant value of $\phi_m (= 0.74)$ are in as good agreement with theory, as in the case of uniform spheres. This indicates that the changes of ϕ_m in respect to the ratios a_2/a_1 and d are relatively small.

So equations (1) and (2) can be safely used at least as approximate expressions in the range covered by numerical experiments. It would be interesting to obtain experimental results for larger concentrations, closer to maximum packing. But it is not clear to me what kind of procedure will be appropriate for generating such concentrations.

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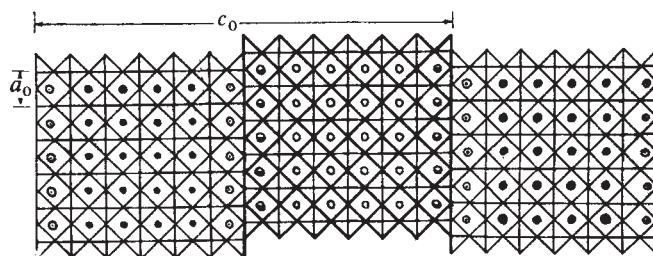
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Lattice images of dislocations in the ferroelectric material $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$

THE compound $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$ is a ferroelectric^{1,2} member of the family of layered bismuth compounds first described by Aurivillius³. These compounds are built up by regular intergrowth of perovskite layers with 'Bi₂O₃' sheets. Between the Bi₂O₃ sheets are m layers of corner-shared octahedra containing the B-cations (Ti⁴⁺, Nb⁵⁺ or Ta⁵⁺) and $(m-1)$ layers of the A-cations (Ba²⁺, Sr²⁺ or Bi³⁺ and so on) in 12-coordinate interstices. A schematic representation of $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$, for which $m = 5$, is shown in Fig. 1. As the ferroelectric properties of these materials are connected with their microstructure it is useful to examine them as closely as possible to elucidate the nature of any disorder present. We report here for the first time the direct observation by lattice imaging of dislocation cores in $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$.

Slightly underfocused lattice images of thin crystals show the actual atomic structure as the projected charge density. By orienting thin crystal fragments so that the electron beam is incident along [100] or [010] of the pseudotetragonal cell, it is possible to obtain lattice images at a resolution of about 3 Å, revealing clearly the positions of the Bi₂O₃ sheets and the perovskite layers. The images show broad, dark lines—the Bi₂O₃ layers, 25 Å apart—with a group of four dark lines

Fig. 1 Schematic projection of $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$, on (010) of pseudotetragonal cell. Fine grid, B-cations at $y = 0$; heavy grid, B-cations at $y = \frac{1}{2}$; ○, Bi at $y = \frac{1}{2}$ in Bi₂O₃ layers; half-filled circle, Bi at $y = 0$ in Bi₂O₃ layers; ●, A-cations at $y = \frac{1}{2}$; ○, A-cations at $y = 0$.



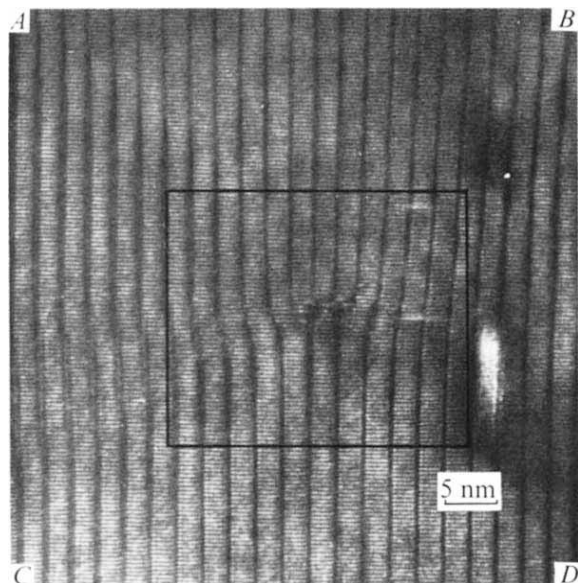


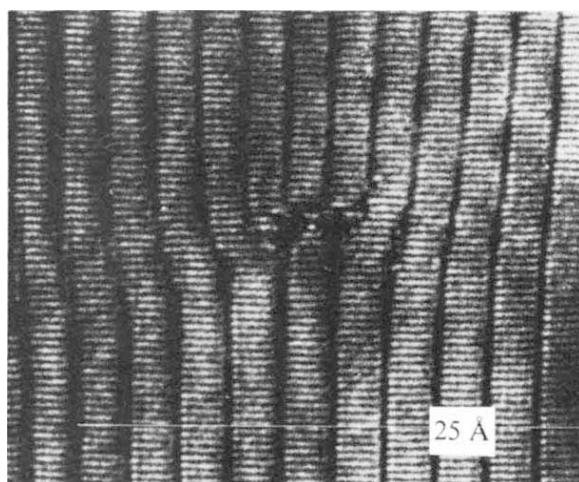
Fig. 2 Lattice image of $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$, containing a dislocation core.

between them, corresponding to the rows of A-cations (Ba and Bi). These cation sites are sometimes resolved as a square grid, and sometimes as lines perpendicular to the Bi_2O_2 layers—the contrast being critically dependent on defocus, crystal thickness, and so on. Figure 2 shows an image of $\text{Ba}_2\text{Bi}_4\text{Ti}_5\text{O}_{18}$ containing a dislocation. There are two extra perovskite slabs and bismuthate layers, corresponding to the insertion of a complete unit-cell of structure. The clarity of the structural detail in the core region (Fig. 3) allows the suggestion that the strain is accommodated in two possible ways.

First, the perovskite layers offer a flexible framework capable of absorbing the compressive strain by distortion of the octahedra. Thus, in the upper part of Fig. 2 there are 23 complete layers (including two extra layers); in the lower part there are only 22 (normal) layers. In the upper part (along A–B) the average width is 2.41 nm, in the lower (along C–D) the average is 2.51 nm, corresponding to $c_0/2$ (refs 1 and 2).

Second, it is possible for the perovskite layers to slip sideways, in units of one (or more) octahedra. This will shift the Bi_2O_2 layers by about 0.4 nm, as shown in Fig. 4. When the A-cations (Ba^{2+} or Bi^{3+}) are resolved parallel to these layers, the jogging

Fig. 3 Enlargement of the area enclosed by rectangle in Fig. 2.



of the Bi_2O_2 layers is clearly visible. Most of the A-cation layers are continuous through these steps, which are also visible in Fig. 3.

The lattice images showed that the crystals contained a very large number of such dislocations, involving one (or more) extra complete layer, although the crystals had been well annealed. This finding may be related to the relatively low Curie temperature for this material (598 K).

The structures of this family of compounds may be described as perovskite lamellae which are in antiphase relationship to

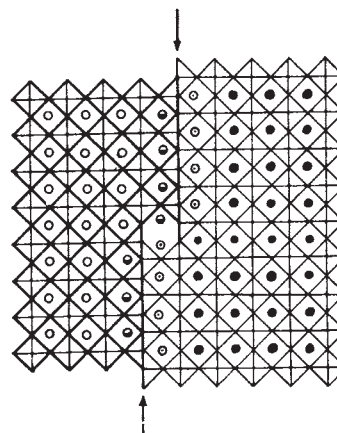


Fig. 4 Idealised drawing of unit shift of 0.4 nm in the Bi_2O_2 layer (indicated by arrows). Symbols as in Fig. 1.

each other, and separated by Bi_2O_2 sheets. The faults described may be regarded as dislocations in the Bi_2O_2 superstructure, imposed on the perovskite sublattice. In order to preserve the antiphase relationship across Bi_2O_2 layers, two extra, complete layers are required in a basal dislocation, as in Fig. 2. In a few cases, only one extra layer is observed; this necessarily involves a dislocation in the perovskite substructure itself. A more detailed analysis of these dislocation cores will be published elsewhere.

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Quality of vacuum-deposited films

THE temperature T_s at which the substrate is maintained during deposition is perhaps the most critical parameter determining the structure and properties of thin solid films produced by the very important techniques of vacuum evaporation and sputtering¹. Apart from situations when T_s becomes so great as to approach the melting point of the film² or to cause complete re-evaporation, the reported variation in properties

Table 1 A summary of substrate temperatures for a variety of vacuum-deposited materials at which optimum properties related to film smoothness/quality have been reported. The optimum temperature T_o is expressed as fractions of the melting point (T_m) and the boiling point (T_b). The vapour pressures at the optima are all calculated to be at least several orders of magnitude below typical evaporator pressures.

Material	Property	Optimum temperature T_o (K)	T_o/T_m (refs for T_m 6, 24, 25)	T_o/T_b	Reference for T_o	Reference for T_b
Anthracene	Smoothness	213	0.44	0.35	—	6
Phthalocyanine	Smoothness	285	—	0.30–0.36	—	*
ZnO	Smoothness, light scattering	768–793	0.34–0.35	0.34–0.36	4	9
Se	Electrophotographic performance	333	0.68	0.35	3, 5	6
ZnTe	Electrical conduction, optical absorption	653	0.43	0.38–0.41	10	11–13
ZnSe	Electro-optic effect	623–643	~0.35	0.34–0.38	14	13, 15–17
PbTe	Hall mobility	523	0.44	0.34	18	19†
CdS	Charge mobility	533	—	0.35	20	21, 22

*Lower limit by equating vapour pressure to that of CuPe (ref. 7); upper limit by including effects of heats of sublimation⁸.

†Also refs cited in ref. 19, neglecting the two extreme values.

as a function of T_s is almost always monotonic. Some properties of a few substances have been shown to be optimal at specified T_s and the smoothness of some epitaxial films have been observed to go through a maximum, but only in the case of poly-crystalline metals does there seem to have been any attempt to systematise these observations.

We report a very clear cut structural singularity in certain organic materials as T_s is varied and show that these and practically all other reported results relating optimum properties of evaporated non-metallic films to substrate temperature occur with T_s very near a constant fraction (0.34) of the boiling point of the evaporated materials. This raises the possibility that a very wide range of materials may show optimum properties near this temperature ratio.

Our measurements have been principally concerned with anthracene films, typically 0.5–1.0 μm thick, which have been evaporated on to thin layers of oxidised aluminium on glass substrates at pressures of approximately 4×10^{-6} mmHg. The anthracene structure is not, however, greatly altered when deposited on other aluminised materials such as mica and melinex and on different evaporated metals. Furthermore, it is not particularly sensitive to the rate of evaporation which has been varied between 15 and 300 nm min^{-1} . Scanning electron micrographs of typical structures obtained at different T_s are shown in Fig. 1. The comparatively smooth structure at -60°C is clearly not part of the normal monotonic variation in crystallite size. Experiments using graded temperature substrates have shown that the optimum temperature range is quite narrow, in our case being within five degrees on either side of -60°C .

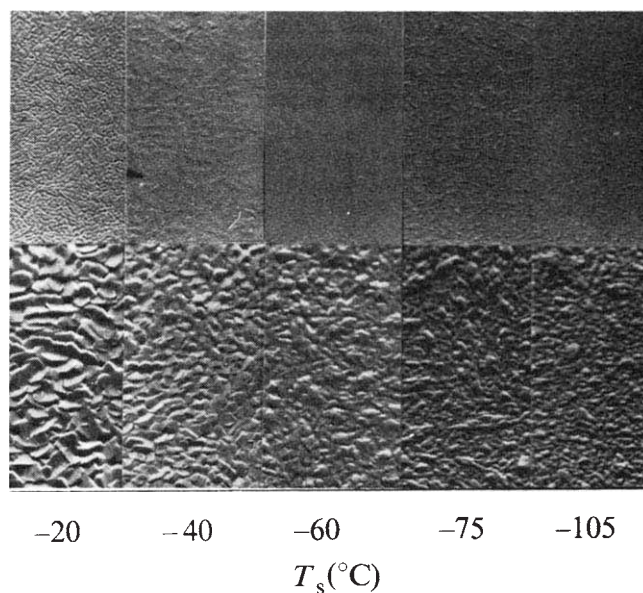
The electrical properties of the anthracene films and their degree of sensitivity to the optimum substrate temperature will be discussed elsewhere. We do report here some interesting electroabsorption data for our samples. Figure 2 shows the change in transmitted light intensity $\Delta I/I$ arising from the application of an electric field as a function of photon energy for a freshly prepared anthracene film evaporated on to a -80°C substrate (for details see ref. 3.). The two distinct peaks (*A* and *B*) that are observed at approximately 430 nm and 393 nm, respectively, are present irrespective of the substrate temperature. Single crystal anthracene exhibits an electroabsorption peak at the latter value (G.G.R., unpublished) and consequently we associate peak *B* with a crystalline arrangement of anthracene molecules. Peak *A* is probably more closely linked with absorption processes involving randomly oriented anthracene molecules. The magnitude of this peak for a constant electric field is a strong function of the substrate temperature. The insert in Fig. 2 illustrates how the ratio of the height of peak *A* to the height of peak *B* (which is relatively insensitive to T_s) varies with T_s . The rapid change

in the region -50°C to -70°C provides strong indirect evidence that a molecular rearrangement occurs near the optimum substrate temperature.

We have obtained similar optima in metal-free phthalocyanine films deposited at 10°C (which no doubt partly explains why this material is well known to form good films on room temperature substrates). A rather similar optimum has been observed⁴ in polycrystalline films of evaporated ZnO at 490–520 $^\circ\text{C}$; this temperature range is the most suitable for integrated optics applications, because excessive light scattering occurs from the rough surface of high temperature films or from the many small particles obtained at low temperatures. Structural factors are also connected^{3,5} with the well known electrophotographic optimum T_s (60°C) for evaporated Se films.

These results are listed in Table 1 together with the optima (T_o) expressed as fractions of the melting point (T_m) and

Fig. 1 Scanning electron micrographs of evaporated anthracene deposited on oxidised aluminium on glass substrates. The temperature T_s of the substrate is indicated. The upper electrode was a thin film of gold deposited on to the structure at -40°C . All the films were deposited at a rate of approximately 30 nm min^{-1} . The magnifications used for the upper and lower sets of photographs were 2 K and 10 K, respectively.



boiling point (T_b). We have also listed other non-metals for which we could find both optima and boiling point or vapour pressure data in the recent literature. We have excluded cases where a specific interaction with the substrate or proximity to a melting point was influencing the optimum. Most of the materials found do not have known boiling points because they dissociate; we have therefore estimated an approximate effective figure by extrapolating vapour pressure data beyond their range of strict validity. Even with this uncertainty the ratios of optimum to boiling point temperatures for almost all the materials we have found, fall within about 10% of 0.34; there is no such correlation with the melting point. For the examples quoted in Table 1 a range of evaporation rates and thicknesses was used. But it may be significant that the example which deviates most from the observed rule is ZnTe which was evaporated at a very fast rate. Accordingly we intend to examine further the influence of evaporation rate and also film thickness on the quality of our vacuum-deposited materials.

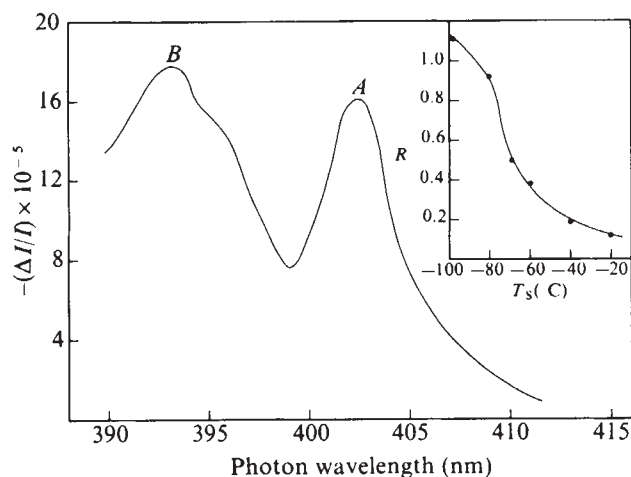


Fig. 2 Fractional change in the transmitted light intensity as a function of photon energy for a freshly prepared anthracene film evaporated on to a substrate held at -80°C . The insert shows the ratio, R , of the electroabsorption peak heights observed at 403 nm and 393 nm as a function of substrate temperature T_s . All data were obtained using an applied electric field value of $5 \times 10^5 \text{ V cm}^{-1}$.

The data of Table 1 provide strong evidence for a close and previously unsuspected relationship between the boiling points and optimum substrate temperatures for a wide range of materials. In most cases it is reported that the surface of the film is smoothest at the optimum temperature. This change in surface quality is probably associated with the occurrence of a structural change in the bulk. Of course several of the film properties involved (such as charge carrier mobility, conductivity and absorption coefficient) are influenced by structural irregularities and therefore a general correlation between these properties and smoothness might be anticipated. It is clear from our electroabsorption studies on anthracene that for this material, the observed smoothness is associated with a transition to a more ordered state. Furthermore it is interesting to note that Genthe and Aldrich¹⁴ have reached a similar conclusion for their results on epitaxial ZnSe films. But, this does not necessarily imply a direct association between the complex mechanism of epitaxial growth and our observed relationship between optimum substrate temperature and boiling point. The optima probably occur because at the given

temperatures newly condensed molecules have just enough energy for limited surface diffusion and reduction of disorder to occur but not enough energy for the substantial movement required for such processes as out of plane crystal growth. The constancy of T_o/T_b is probably related to the tendency for the migration energy of an adsorbed molecule to be about one third of its binding energy in the bulk¹. Our observations suggest that other materials may show optima for $T_s/T_b \sim 0.34$, but there are also materials in which substantial ordering processes can occur without the degree of molecular freedom present at these temperatures; this is presumably the reason, for example, why resistivity and density optima in metals²³ take place at considerably lower values of T_s/T_b . The possibility remains that other optima may be seen at ratios of 0.34 even in these materials.

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Oxidation of nickel by nitric oxide as a new strong oxidant

We have found that nitric oxide (NO) behaves as a strong oxidant for nickel at high temperatures. Oxidation curves of nickel plates were measured in nitric oxide (700°C , 10 mmHg) and in oxygen (700°C , 10 mmHg) by means of a microbalance and the results are shown in Fig. 1. A linear rate law was observed for nitric oxide but a parabolic rate law for oxygen where the rate of oxidation was almost the same as that published¹. The rate of the oxidation of nickel in nitric oxide was very rapid in comparison

with that in oxygen. The X-ray diffraction patterns from the scales formed on both of these samples gave only the patterns of NiO, and the ion microanalysis (IMA) spectra showed no evidence of nitrogen in these scales.

Figure 2 shows the temperature dependence of the rate constant of the oxidation of nickel at 10 mmHg of nitric oxide. The apparent activation energy of the oxidation in nitric oxide was $17.8 \text{ kcalorie mol}^{-1}$, less than that in oxygen ($27.7 \text{ kcalorie mol}^{-1}$).

In Fig. 3, the effect of the pressure on the rate constant of the oxidation of nickel in nitric oxide at 700°C is shown. From this relationship, the reaction order is found to be 0.74 in contrast to the value of 0.16 generally found for the oxidation of nickel in oxygen^{2,3}. These findings suggest that the oxidation of nickel in nitric oxide is governed by a surface reaction, whereas that in oxygen is governed by diffusion of the metallic ions through the oxide layers⁴.

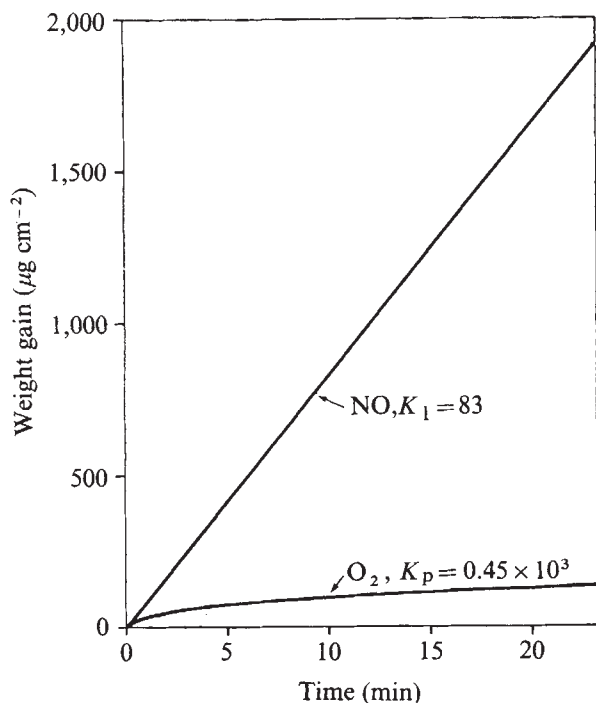


Fig. 1 Oxidation of Ni in nitric oxide or oxygen at 700°C and 10 mmHg. K_1 ($\mu\text{g cm}^{-2} \text{ min}^{-1}$), linear rate constant; K_p ($\mu\text{g}^2 \text{ cm}^{-4} \text{ min}^{-1}$), parabolic rate constant.

Fig. 2 The relationship between K_1 and temperature ($P_{\text{NO}} = 10 \text{ mmHg}$). $E = 17.8 \text{ kcalorie}$.

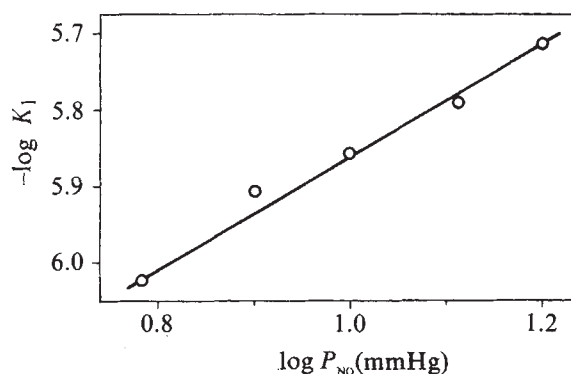
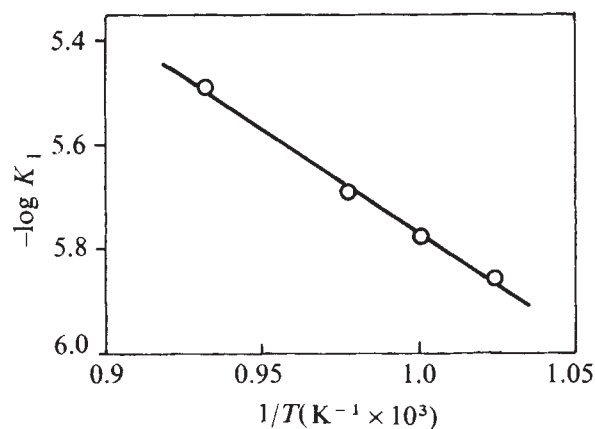


Fig. 3 The relationship between K_1 and the pressure of nitric oxide (700°C). $n = 0.74$.

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Epidemic typhus rickettsiae isolated from flying squirrels

EPIDEMIC typhus is a severe rickettsial infection that classically has involved only man and his body louse. Although the aetiological agent, *Rickettsia prowazekii*, has been isolated from ticks of domestic animals and from blood of livestock in Ethiopia and Egypt^{1,2}, infection of these unusual hosts and vectors has been thought to be secondary to active dissemination of the louse-borne disease in the human population³. Ormsbee⁴ concluded that livestock and the equivalent wild species were unlikely to be important in the ecology of epidemic typhus⁴. This disease was last reported in the eastern United States in Philadelphia in 1836 (ref. 5). We have now isolated six strains of *R. prowazekii* from the eastern flying squirrel (*Glaucomys volans volans*). This finding suggests that an extrahuman reservoir of epidemic typhus can exist in a species of wild rodent independent of the recent occurrence of epidemic or sporadic disease.

Serological evidence of this enzootic typhus group infection in flying squirrels was first found near Montpelier, Virginia in 1963. Complement fixation (CF) tests, using species-specific corpuscular antigens prepared from yolk sacs infected with epidemic and murine typhus rickettsiae and *R. canada*, were carried out on specimens collected in 1968. The results showed that, contrary to previous assumption, *R. typhi* was not the causative organism, but that it was either *R. prowazekii* or *R. canada* or another organism antigenically related to both.

CF tests with sera from 30 more squirrels collected near Ashland, Virginia in the spring of 1972 showed that 42% of the animals were infected. During the autumn and winter of

Table 1 Data relevant to isolation of epidemic typhus rickettsiae from flying squirrels from Florida

Gv No.	1st serum	Serology		Tissues inoculated into	Results of isolation attempts	
		IF titre* (d after arrival in laboratory)	2nd serum		IF titre when killed	Rickettsiae isolated in eggs
F-28	< 10†(12)	2,560 (19)	2,560 (27)‡	GvF-8	2,560 (15)§	Yes
F-31	< 10 (12)	640 (19)	640 (28)‡	GvF-12	640 (14)	Yes
F-38	< 10 (12)	10 (19)	2,560 (27)‡	GvF-16	2,560 (15)	Yes
F-75	< 10 (6)	10 (12)	10 (13)‡	GvF-18	2,560 (13)	Yes
F-82	< 10 (6)	10 (12)	10 (13)‡	GvF-24	2,560 (13)	Yes
F-134	< 10 (1)	2,560 (8)	—	Eggs	—	Yes

*Final titre was the highest dilution of serum that gave 1+ fluorescence on a scale of 1 (minimal) to 4 (maximal) on antigen smears of infected yolk sac suspensions of epidemic typhus rickettsiae standardised so that there were approximately 1,000 organisms per high-power field. Serial fourfold dilutions (1:10–1:2,560) of serum were used. Anti-*Glaucomys volans* (Gv) fluorescein isothiocyanate-labelled globulin was prepared from serum from rabbits immunised with flying squirrel serum globulins. When typhus group antibodies were found, either in animals just received or after conversion in the laboratory, in almost every instance the IF titre with *R. prowazekii* was higher than with the *R. typhi* and *R. canada* antigens. *R. rickettsii* and *C. burnetii* antibodies were not found in any of the flying squirrels.

†Reciprocal of serum dilution.

‡Day animal killed.

§Day after inoculation.

1972–73, 20 squirrels were trapped in this area and killed. Attempts to isolate rickettsial agents from suspensions of spleen, liver and kidney inoculated into embryonated hens' eggs and into guinea pigs were negative.

To avoid disturbing the ecological balance in the Ashland area, animals were obtained from a commercial source in Florida. Between December 1973 and July 1974, 215 flying squirrels were received, all recently captured in wooded areas in northern and central Florida. Within a few days after

for 3 weeks and at 2-month intervals thereafter. In 27 of the negative squirrels, seroconversions, indicated by the presence of antibodies in subsequent specimens, were noted and 15 of these animals were killed. A 20% w/v suspension of liver, kidney and spleen homogenised in Snyder I diluent⁷ was prepared from each animal. The first 13 suspensions were each inoculated intraperitoneally into a previously confirmed seronegative squirrel, and the other two directly into the yolk sac of 6–8-d-old embryonated eggs (SPAFAS, Inc.). Five of

Table 2 Relationship of rickettsiae isolated from flying squirrels to the members of the typhus group as revealed by CF

Guinea pig antiserum	Complement-fixing antibody* titre with 4 U of specific rickettsial antigen				
	GvF-12	GvF-16	<i>R. prowazekii</i>	<i>R. typhi</i>	<i>R. canada</i>
GvF-8	640†	640	320	< 160	< 160
GvF-12	640	640	640	< 160	< 160
GvF-16	320	320	320	80	< 80
GvF-18	320	640	320	< 160	< 160
GvF-24	640	640	320	< 160	< 160
GvF-134	320	320	320	< 80	< 80
Epidemic typhus	256	256	256	64	< 64
Murine typhus	64	64	64	256	< 64
<i>R. canada</i>	< 16	< 16	< 16	< 16	64

*CF tests were performed according to the LBCF microtitre method¹⁰.

†Reciprocal of serum dilution.

arrival they were bled by the orbital technique⁶. Their sera were tested by indirect immunofluorescence (IF) for antibodies to *R. prowazekii* (Breinl strain), *R. typhi* (Wilmington), *R. canada* (2678), *R. rickettsii* (Sheila Smith) and *Coxiella burnetii* (Henzerling). When first bled, 104 squirrels had typhus group antibodies, that is, their sera had an IF titre of 1:10 or greater. Seronegative and seropositive squirrels were housed in an isolated room in different cages, usually in groups of six. The 111 negative animals were bled again at 6–10-d intervals

the inoculated squirrels developed typhus group antibodies within 12–14 d; each was killed and a pool of its tissues was inoculated into eggs as previously described. Table 1 shows the recovery of rickettsiae from these animals. Another strain was recovered by direct inoculation of eggs with tissues from a squirrel that seroconverted 8 d after arrival. The other attempt at direct isolation using eggs was unsuccessful. Each strain was reisolated in eggs from samples of the corresponding tissue suspensions that had been stored for 5–6 months. The classical

Table 3 Relationship of rickettsiae isolated from flying squirrels to the members of the typhus group by toxin neutralisation

Antiserum	50% Toxin neutralisation titre* with rickettsial suspensions			
	GvF-16	<i>R. prowazekii</i>	<i>R. typhi</i>	<i>R. canada</i>
GvF-16 guinea pig	407†	407	≤ 11	≤ 11
Epidemic typhus guinea pig	204	362	16	≤ 11
Murine typhus guinea pig	45	16	512	≤ 11
<i>R. canada</i> rabbit	≤ 11	≤ 11	≤ 11	16
Virginia flying squirrels	512	362	≤ 11	< 11

*Twofold dilutions of serum were mixed with 4 mouse LD₅₀ of toxin and incubated at room temperature for 1 h. Groups of four mice were injected intravenously with 0.5 ml of the toxin-serum mixture. Deaths were recorded up to 18 h. 50% Serum neutralisation endpoints were determined by the method of Reed and Muench.

†Reciprocal of serum dilution.

rickettsial strains were never handled on the same day that isolation procedures were carried out, and all autopsies and processing of tissues and eggs were done in a biohazard laminar flow hood.

The egg 50% lethal dose (LD_{50}) titres of early passage seed suspensions of each of the strains ranged from $10^{3.6}$ to $10^{6.0}$; the 50% infectious dose (ID_{50}) titres, determined by examining stained smears of yolk sacs of embryos surviving for 12 d, were 10–500 times higher.

Adult Swiss mice inoculated intraperitoneally with 10% yolk sac suspensions died within a few hours as a result of rickettsial toxin. Suspensions diluted beyond lethal levels caused no apparent disease, but mouse ID_{50} titres based on serological conversion were essentially the same as those found in eggs. Serial twofold dilutions of suspensions of the different strains inoculated intravenously into mice had toxin LD_{50} titres varying from 1:64 to 1:256.

Soluble complement-fixing antigens⁸ prepared with the isolated strains were group-reactive in tests with guinea pig, rabbit and mouse immune sera.

Species-specific corpuscular antigens⁹ prepared from two strains titred 1:256–1:320 with homologous antibody. The results of grid-type CF tests with these antigens and the other members of the typhus group and corresponding guinea pig sera are shown in Table 2. Thus the rickettsial agents isolated from flying squirrels were closely related, if not identical to *R. prowazekii*. Toxin neutralisation (TN), another definitive method for species identification, showed that the strains were indistinguishable from *R. prowazekii* (Table 3). The results of the TN test with a pool of sera from animals caught in Virginia that had IF titres of 1:640 and 1:2,560 suggest that the rickettsial agent is enzootic also in Virginia.

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into the aggregation phase coincides with an increase in the number of specific contact sites (known as contact sites A; ref. 3) on the surface of the cells. The appearance of contact sites A is thus an index of differentiation and we have used it to test the hypothesis that cyclic AMP is a signal for initiating differentiation^{6,7}, as well as acting as the aggregation signal for differentiated cells.

Cyclic AMP is spontaneously released by *D. discoideum* cells before the development of full aggregation competence. The release of cyclic AMP is pulsatile^{8,9} and there is evidence to suggest that the chemoreceptor system underlying aggregation responds to the rapid increase in cyclic AMP concentration with time, rather than to the concentration *per se*¹⁰. We therefore used a pulsatile cyclic AMP signal to stimulate differentiation in stationary phase cells. In contrast to cells harvested at the exponential growth phase, differentiation is blocked in these cells. This block can be bridged by cyclic AMP pulses (Fig. 1). Continuous flow application of cyclic AMP at the same average rate had no significant effect on differentiation. The size of the stimulatory pulses (5 nmol per 10^{10} cells) is much lower than the amplitudes of the periodic cyclic AMP spikes which are spontaneously generated by differentiating cells (50–100 nmol per 10^{10} cells). Since it is known, however, that extraneous cyclic AMP pulses can stimulate cyclic AMP release from the cells, so that they are amplified by one to two orders of magnitude^{8,11}, the effective pulse sizes may be close to each other in both conditions.

The formation of EDTA-stable cell contacts is a specific function of contact sites A. To show that the control of these sites by cyclic AMP pulses is based on their accelerated appearance at the cell surface, rather than on the control of their activity, the antigenic expression of contact sites A at the cell surface was measured by F_{ab} binding. Figure 2 shows enhanced expression and function in response to pulsatile signals.

Contact sites A did form in non-stimulated stationary phase cells, as well as in the same cells exposed to continuous flow of cyclic AMP, but there were fewer and they appeared more slowly (Fig. 2). Cell adhesion measured in the presence of EDTA indicated that these sites, when formed, also become active (Fig. 2). Whereas in exponential phase cells the appearance and activity of contact sites A is largely correlated with the ability of the cells to elongate and to adhere preferentially at their ends, both functions are dissociated in stationary phase cells (compare Figs 1b and c and 2), indicating strict dependence of cell elongation on stimulation by cyclic AMP pulses. Actin is the predominant protein synthesised in the interphase between growth and aggregation¹². One can speculate that a high rate of actin synthesis is necessary for the change of cell shape, and that this synthesis is under the control of cyclic AMP pulses.

Exponential phase cells showed only slight acceleration of differentiation, which was advanced by about 1 h in response to pulses of 2×10^{-8} M cyclic AMP per 10 min. In these cells the experimental cyclic AMP pulses on one hand induce premature onset of spontaneous pulsing, but on the other interfere with established oscillations, and the results may critically depend on the balance of these actions. Continuous flow at the same average rate delayed differentiation by 4 h, so that the cells were not fully differentiated after a period of 9 h.

Continuous-flow application of cyclic AMP is known to suppress spontaneous oscillations¹⁰. The inhibition of cell differentiation by continuous flow suggests that in cells collected at the exponential phase, differentiation is self-stimulated by the periodic pulse generating system, and that the continuous flow interferes with but does not mimic its action on cell differentiation.

If differentiation is self-stimulated by the cyclic AMP which the cells release, it should be delayed by cyclic-AMP

Control of cell-contact sites by cyclic AMP pulses in differentiating *Dictyostelium* cells

SINGLE cells of the slime mould *Dictyostelium discoideum* differentiate after the end of the growth phase of their life cycle into interacting cells which form aggregates by chemotaxis towards a source of cyclic AMP^{1,2}, with the formation of specific contacts between cells^{3–5}. Emergence

phosphodiesterase. To test this hypothesis extracellular phosphodiesterase of *D. purpureum* was used because this enzyme is largely resistant to the phosphodiesterase inhibitor produced by *D. discoideum* cells⁶. Consequently, constant activities could be maintained over the whole

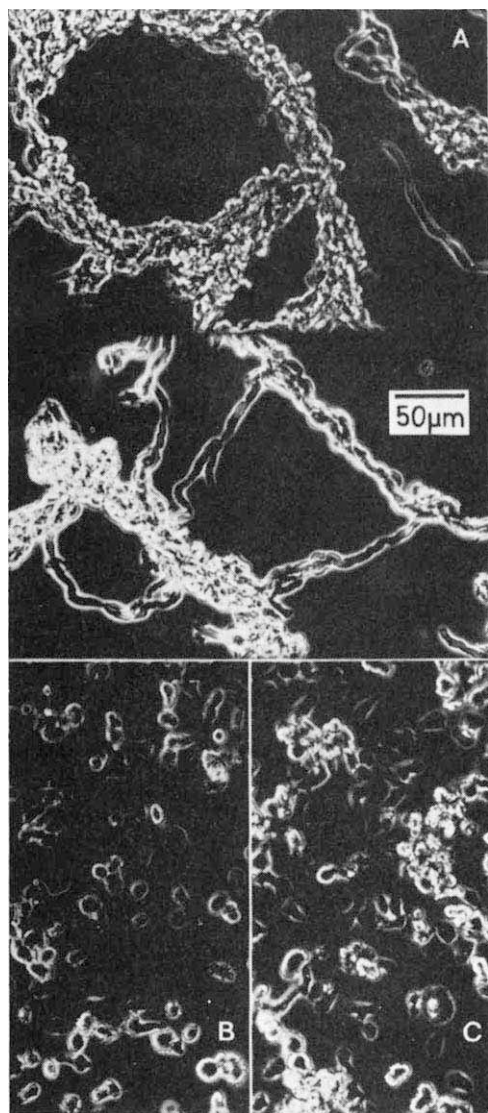


Fig. 1 Effect of cyclic AMP pulses on differentiation of cells collected at the stationary phase. **A**, Pulse-stimulated cells reached full aggregation-competence within 8 h, as indicated by extensive elongation of the cells, their end-to-end adhesion, and their ability to aggregate into whirl-like aggregates (top) and stream-like assemblages (middle). After steady-flow application of cyclic AMP (**C**) the cells did not differ from control cells (**B**). They were not elongated, but tightly sticking to the glass and only partially aggregated into irregular clumps. Cells of the axenic strain Ax-2 of *D. discoideum* were cultivated on nutrient medium supplemented with 1.8% maltose²², and collected at a density of $2.0 \times 10^7 \text{ ml}^{-1}$, washed and adjusted to $1.0 \times 10^7 \text{ ml}^{-1}$ in 0.017 M phosphate buffer pH 6.0. $3 \times 10^{-6} \text{ M}$ cyclic AMP (**A**, **C**) or buffers (**B**) were fed into 300-ml cell suspension at a constant rate of 0.380 ml h^{-1} , beginning about 40 min after resuspension. In **B** and **C** the material was immediately injected into the cell culture, in **A** it was first collected on a microbalance and dropped into the culture in volumes of approx 40 μl . The mean period of dropping was 6.5 min. Because samples had to be taken for the measurements shown in Fig. 2, the mean amplitude of the pulses increased in **A** from 4.2 nM cyclic AMP at the beginning to 6.4 nM at the end of the experiment. Accordingly, in **C** the mean rate of injection increased from 0.63 to $1.0 \text{ nmol min}^{-1} \text{ l}^{-1}$. Cells were removed from the suspension 8 h after washing, and allowed to aggregate for 2 h in the absence of any stimulation before photographs were taken.

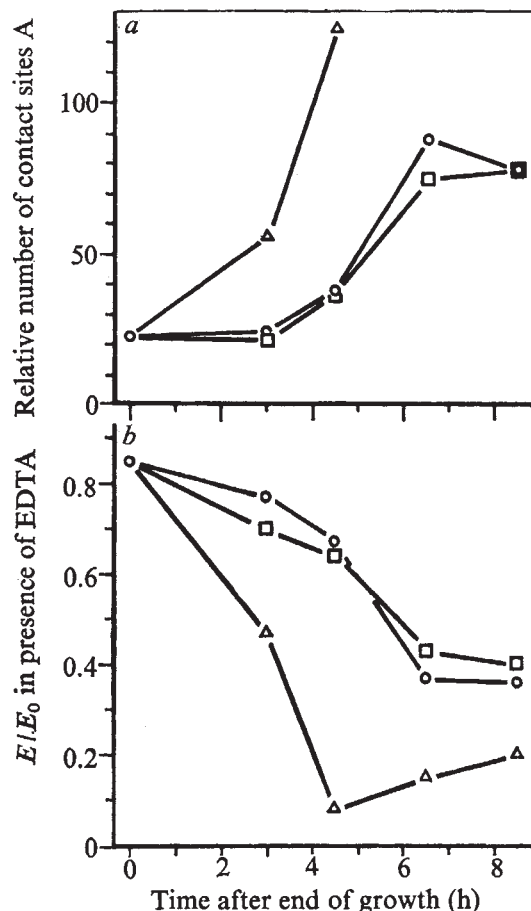


Fig. 2 Exposure of contact sites A at the cell surface (**a**) and EDTA-stable cell adhesion as a measure for the activity of these sites (**b**). Δ , Cells stimulated by cyclic AMP pulses corresponding to Fig. 1A; $\circ$, control according to Fig. 1B; $\square$, continuous cyclic AMP application as in Fig. 1C. **a**, Contact sites A were measured by absorption of F_{ab} from antiserum directed against aggregation-competent cells. The aggregation-inhibiting activity of the unabsorbed F_{ab} was then retitrated in the presence of 10 mM EDTA using aggregation-competent cells as described in ref. 3. The ordinate value of 100 corresponds to the relative number of contact sites A found in differentiated Ax-2 cells 9 h after collection at the exponential phase. The low background absorbing activity corresponding to a relative number of approximately 20 contact sites A found in non-differentiated cells, is the result of contact sites B which are already present in growth-phase cells and which are not fully inactivated by the EDTA used. Absence of contact sites A from non-differentiated cells has been demonstrated by use of F_{ab} from which the anti-B activity had been removed³. **b**, EDTA-stable cell adhesion. Ordinate: E/E_0 refers to light scattering of the test sample, E_0 of an identical single-cell suspension. Low E/E_0 values indicate strong aggregation in the presence of 10 mM EDTA in 0.017 M phosphate pH 6.0. Abscissa: time after separation of the cells from the nutrient medium, as outlined in the legend to Fig. 1. All measurements for **a** and **b** were made after washing the cells three times so that the different nucleotide composition of the culture fluids could not directly influence the results.

period of cell differentiation to aggregation-competence. The strain used was the fast differentiating wild-type clone M2 grown on bacteria; $10^7 \text{ cells ml}^{-1}$ were suspended in 0.017 M phosphate buffer pH 6.0. In these conditions, relatively high phosphodiesterase activities of 40–80 $\text{nmol min}^{-1} \text{ ml}^{-1}$ were required to delay cell differentiation; and only in one of three experiments was the delay more than 1 h¹³. (The enzyme activities are those calculated for the experimental conditions; they have to be multiplied by a factor of 5 to get the standard activities measured at 35 °C pH 7.4, as published previously⁶.) The low efficiency of phosphodiesterase in delaying cell differentiation is easily explained if one imagines that for the cyclic AMP released during a pulse, a half life of about 2 s is sufficient to

activate the receptors^{9,14} and that the enzyme, by lowering the background level of cyclic AMP, could even increase the sensitivity of cells to pulses. We cannot strictly rule out the possibility, however, that the dependence of cell differentiation on extracellular pulses is a peculiarity of axenically grown cells collected at the stationary phase, whereas in other cells the extracellular loop of the signal system is redundant. In this case, the intracellular oscillation of metabolites could be the key for cell differentiation. During an oscillatory cycle, the intracellular cyclic AMP concentration changes by a factor of 10 with peak values of 20 μ M.

It is possible to interpret these results in terms of a receptor system which responds to a rapid increase of cyclic AMP concentration in time. A receptor system of this type would also account for the observation that, on an agar surface, cyclic AMP has to be applied in a spatial gradient to induce cell adhesion¹. When guided by chemotaxis, the cells migrate towards higher concentrations, so that the cyclic AMP concentration at their surface automatically increases with time.

The response to concentration steps would relate the morphogenetic signal system in *D. discoideum* to other sensory systems exhibiting an 'on'-response. Examples are the phototropic response of *Phycomyces*¹⁵, phototaxis in colonial green algae¹⁶ and chemotaxis in bacteria^{17,18}. These results underline the possibility that not only the time pattern of signalling¹⁹, but also the dynamics of the receptor system, could be an important parameter for the action of morphogens in controlling embryogenesis. In relation to neurodevelopment it may be of interest that, in *Dictyostelium*, the formation of adhesion sites operating in specific cell connectivity is facilitated by the action of a low molecular weight transmitter which, released in periodic pulses, passes an intercellular gap. Oscillations of acetylcholine in stimulated electroplax tissue²⁰ parallel the cyclic AMP oscillations found in *Dictyostelium* and suggest similar control mechanisms^{21,23} for the transmitter synthesising enzymes in both systems.

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Secretion of cyclic AMP induced by cyclic AMP in the cellular slime mould *Dictyostelium discoideum*

A STRIKING feature of aggregation in large species of *Dictyostelium* is the appearance of repeated waves of centripetal movement propagated outwards from the centres^{1,2}. I accounted for them as follows³⁻⁸. The cells are not only oriented by the chemotactic agent, acrasin⁹, but induced to secrete it; they thus relay the orienting signals. The detailed aggregation pattern, before contact modifies it, records the transmission of these signals. To avoid an infinite regress, some secreted factor must induce its own secretion; this was tentatively assumed to be the attractant itself. There would then be separate thresholds for attraction and secretion. The dual responsiveness develops during the preaggregation phase. Aggregation centres are started by the first cells to secrete signals supraliminal for their neighbours. That separate cells, having oriented to the signal and transmitted it outwards, do not reorient to the signal now coming from behind them and transmit it centripetally, was ascribed to some combination of reduced receptor, motor, and secretory activities, expressed as an absolute or relative refractory period, and to changes in cell shape, spacing, orientation, and polarity, once the cells have responded. The precise signal profile may be significant. After an interval, the cells can transmit a new pulse in any direction. Commonly the same cells as before initiate the later pulses and thus remain dominant, but pulses from other pacemakers can recruit and reorient those cells they reach first. Secreted acrasin is rapidly inactivated enzymically. It was argued that this is functionally useful in maintaining the detectability of the signal even with a single source, and vastly more so with literally millions of sources. Enzymic breakdown could also yield a metabolite for recycling. Later work¹⁰⁻¹² has very largely confirmed this. The attractant in *D. discoideum* is cyclic AMP¹³, and the inactivating enzymes phosphodiesterases (PDE)¹⁴⁻¹⁶. Cyclic AMP pulsed from a microelectrode induces propagated waves of movement¹⁷. A mathematical model has incorporated most of the above features¹⁸. The present work demonstrates directly that cyclic AMP does induce aggregating cells to secrete cyclic AMP.

Although cells in suspended culture become aggregation-competent¹¹, normal aggregation will occur only on a surface, and the standard method for studying the biochemistry of aggregation has been to let the amoebae develop on dialysis membrane, Millipore membrane, or filter paper, and analyse the underlying fluid^{4,19}. Cyclic AMP output has been measured at intervals of several hours²⁰. Added 10⁻⁴ M cyclic AMP has been shown to increase Ca²⁺ secretion, measured after 10 min (ref. 21), and most pertinently, actually to decrease cyclic AMP secretion 20-fold, measured after many hours²².

A quicker method was needed. The cells were made to develop not at an air interface on agar or some fabricated material, but on glass under water. Such cells aggregate normally⁹, but the maximum densities are much lower. To compensate, a large perfusion chamber was devised from an optically flat 9-cm Petri dish (Anumbra). The bottom, with a 2-mm diameter hole drilled in its centre, was stood, right way up, inside the inverted lid. The flat glass surfaces were kept 150 μ m apart by 3 1-mm² Sellotape spacers, and thus enclosed ~0.95 ml of culture fluid, which during perfusion was withdrawn through the central hole and replaced by fluid added at the periphery in the gap between lid and bottom.

D. discoideum NC4 was grown on glucose-peptone agar⁹ with *Escherichia coli* at room temperature, and collected near the

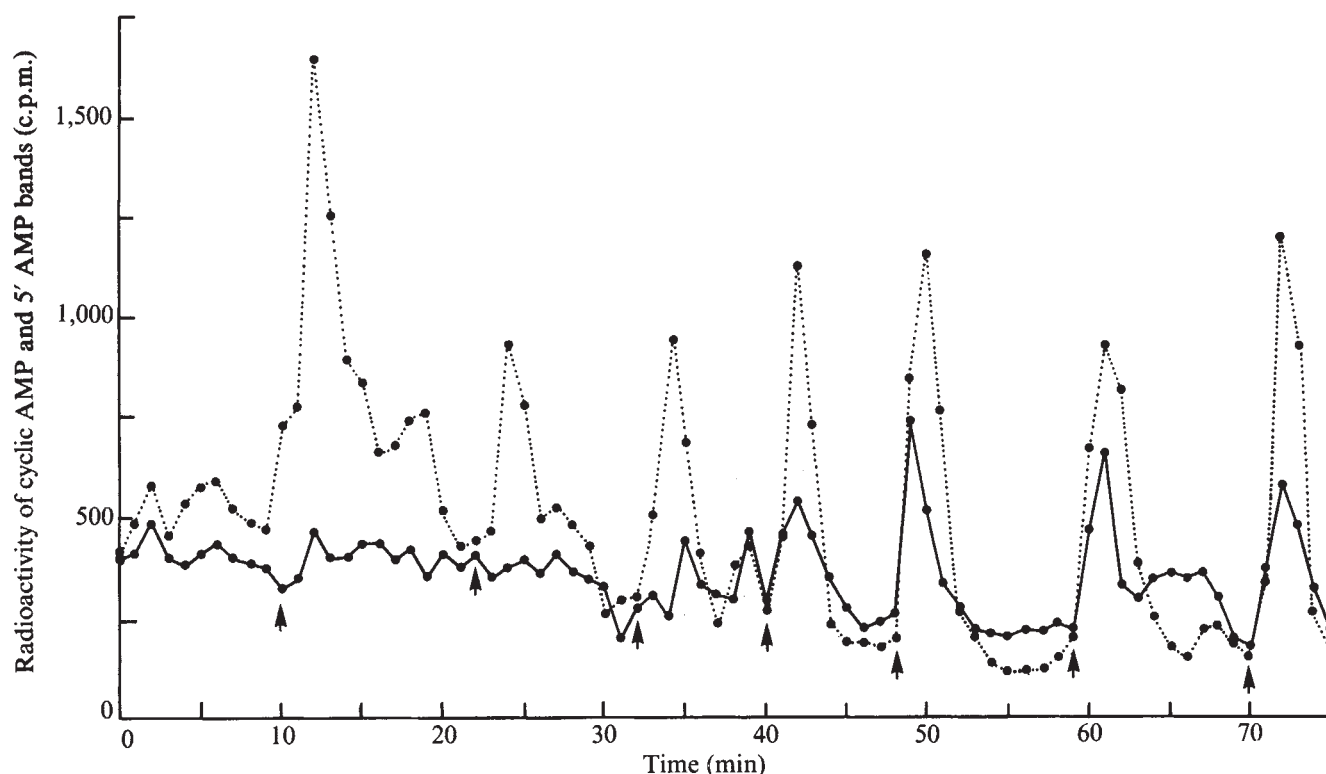


Fig. 1 Radioactivity of the cyclic AMP and 5'-AMP bands from IBAA chromatography of the perfusion fluid of ^3H -labelled *D. discoideum* cultures bathed with unlabelled 10^{-6}M cyclic AMP (arrows). Cells deposited at $1,500\text{ mm}^{-2}$ with ^3H -adenine and kept stationary. Stippled aggregation after 9 h; cultures then washed and perfused at 20°C . —, Cyclic AMP band; ····, 5'-AMP band. The 5'-AMP band peaks after the first and each subsequent perfusion with cyclic AMP: the cyclic AMP band peaks only after the later perfusions. The apparently anomalous small peak in both bands at 39 min could well have followed the late entry into the chamber of one drop of cyclic AMP temporarily trapped between the rims of the dish.

end of vegetative growth by suspending the cells in ice-cold distilled water. In almost all previous work, such cells have been freed from bacteria by repeated centrifuging⁹, but I have found this unnecessary. Instead, the cells were simply allowed to settle in the inverted Petri dish lid, half full of diluted (10 times) Bonner's saline⁹, containing 6 mg streptomycin l^{-1} , to give a density of $1,500\text{--}2,500$ cells mm^{-2} , measured directly with a microscope after spreading. The outermost 5 mm was swept clear of cells. Two washes with saline removed virtually all the bacteria. In most experiments (Fig. 1), the cells were then left in 5 ml of saline containing $100\text{ }\mu\text{Ci}$ ^3H -8-adenine (17 Ci mol^{-1}) for 8–12 h at room temperature until the majority had entered 'stippled' aggregations: large, rather indefinite fields, in which the cells were elongated and relayed the pulses, but had yet to make extensive contact⁷. This stage was chosen because older cells, in compact streams, tended not to respond to natural external acrasin stimuli⁶. But some cells had already entered more compact aggregations, whereas others were still unaggregated.

The cells were washed three times with saline, left at least 30 min, and washed again. The original bottom of the Petri dish was placed inside the lid, and the surplus fluid sucked up through the central hole until none remained beyond the area of flat, parallel glass. Each minute, 1 ml of fresh saline was added around the periphery in four equidistant aliquots in the period –15 to –5 s, and 1 ml was withdrawn from 0–10 or 0–15 s. At least 20 1-min withdrawals were discarded. Those to be collected were expelled into vials through an AA Millipore filter, which removed any detached cells, and kept on ice. The culture was perfused with $1\text{ ml } 1 \times 10^{-6}\text{ M}$ cyclic AMP in saline at intervals of 6–12 min. The vials were stood in boiling water for 2 min to inactivate PDE and stored frozen. The contents were dried down and cochromatographed with cyclic AMP, 5'-AMP, ATP, ADP, adenine and adenosine on thin-layer cellulose plates with isobutyric acid–ammonia (0.88)–

EDTA (0.1 M)–water, 100:42:1.6:55.8 (IBAA). Bands were scintillation-counted in toluene–Triton X-100 (2:1) containing 0.8% butylphenylbiphenyloxazole.

Peaks of labelled material were found in the band containing cyclic AMP and the band containing 5'-AMP (Fig. 1). But whereas the 5'-AMP band peak appeared after the first and each subsequent perfusion with cyclic AMP, the first appearance of the cyclic AMP band peak was variable. In the Fig. 1 experiment, it appeared clearly only after the fourth cyclic AMP perfusion, doubtfully after the third; in some other experiments, after the second or first.

PDE could readily account for the 5'-AMP peaks accompanying peaks of secreted cyclic AMP. The unaccompanied 5'-AMP peaks may be explained by the presence of more PDE before extensive perfusion, or by slight residual extracellular PDE activity at 0°C . These explanations seemed challenged by the rather high but fairly constant activity in the cyclic AMP band before its peaks appeared, but were still tenable if this label was not in fact in cyclic AMP. The cyclic AMP band from a continuation of the Fig. 1 experiment was eluted from the IBAA plates with distilled water and electrophoresed on cellulose plates in 50 mM phosphate buffer at pH 8. There remained in the cyclic AMP band very marked peaks of cyclic AMP-induced activity, but virtually no detectable activity between peaks. The substantial amount of label that moved towards the cathode was much more constant in successive minutes and unaffected by the applied cyclic AMP (Fig. 2); it may well have been hypoxanthine, which was found to move in almost the same position in these treatments. There was no significant activity elsewhere.

That the label in the cyclic AMP band after electrophoresis was indeed in cyclic AMP was established by re-eluting it with distilled water, pooling the material from several peaks, and incubating with 3',5'-cyclic nucleotide PDE (Sigma), Tris-buffered at pH 7.5 in the presence of Mg^{2+} . After further

electrophoresis, as before, all detectable activity was found in the 5'-AMP band and none in the cyclic AMP position.

In later experiments, once the cells had attached to the glass, they were swirled until perfusion time in 8 ml saline and 100 μ Ci 3 H-adenine on a rotary shaker at 50 r.p.m. This not only improved developmental synchrony, but by interfering with the formation of gradients, retarded the appearance of compact aggregations by several hours. Nearly the whole dish became covered with separate, elongated, 'aggregating' cells, mostly lacking any overall orientation: an ideal condition. Also, each collecting vial contained 0.1 ml 0.1 N formic acid, bringing the culture fluid to pH 3, at which PDE was inactive. In the Fig. 3 experiment, both cyclic AMP and 5'-AMP bands were eluted from IBAA plates, and separately electrophoresed as before. This did not appreciably purify the 5'-AMP band material. Its identity with 5'-AMP was tested by eluting several 5'-AMP band peaks from IBAA plates with distilled water and incubating the pooled eluate with 5'-ribonucleotide phosphohydrolase (EC 3.1.3.5, Sigma) at pH 8, which hydrolyses 5'-AMP to adenosine. After IBAA chromatography, 80% of the label was found in the adenosine band. In the Fig. 3 experiment, cyclic AMP pulses were induced from the first application of cyclic AMP. Also, the 5'-AMP peaks lagged behind the cyclic AMP ones, whereas in earlier experiments they were coincident. This difference may possibly have been caused by hydrolysis in the culture dish no longer being obscured by hydrolysis after collection.

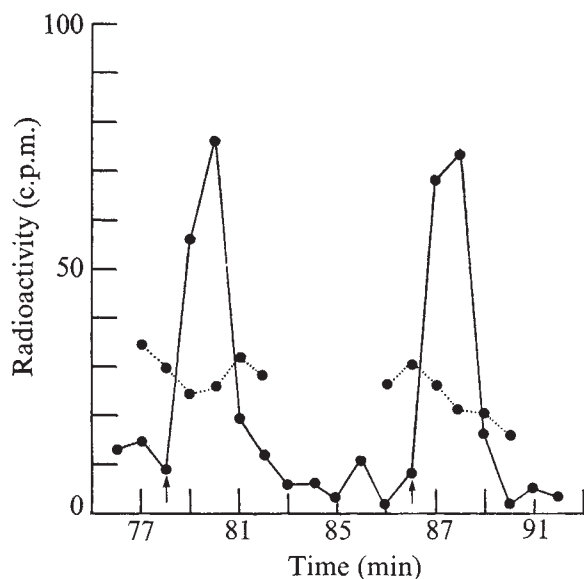


Fig. 2 Later material from experiment in Fig. 1 further purified. 10^{-6} M cyclic AMP added as shown by arrows. Cyclic AMP band from IBAA chromatography eluted with 1 ml distilled water. 0.3 ml eluate electrophoresed in 50 mM phosphate buffer pH 8.0. —, Radioactivity in cyclic AMP band; ·····, in negatively moving material tentatively identified as hypoxanthine.

Apart from the actual existence of the peaks, the most striking feature was their extended time course. Certainly the culture chamber allowed appreciable mixing at each perfusion, so that not all the added cyclic AMP was washed out after 1 min; nor was all the label secreted in any 1 min collected at the next withdrawal. Nevertheless, this alone could not readily account for cyclic AMP activity often being, as in Fig. 3, several times greater in the minute following removal of most of the added cyclic AMP. The individual cell responses may have been much shorter, and the lengthy response a cascade triggered by the applied cyclic AMP. Added 1×10^{-5} M, 1×10^{-4} M, and 1×10^{-3} M cyclic AMP, however, induced similar but not quicker responses. If the observed response

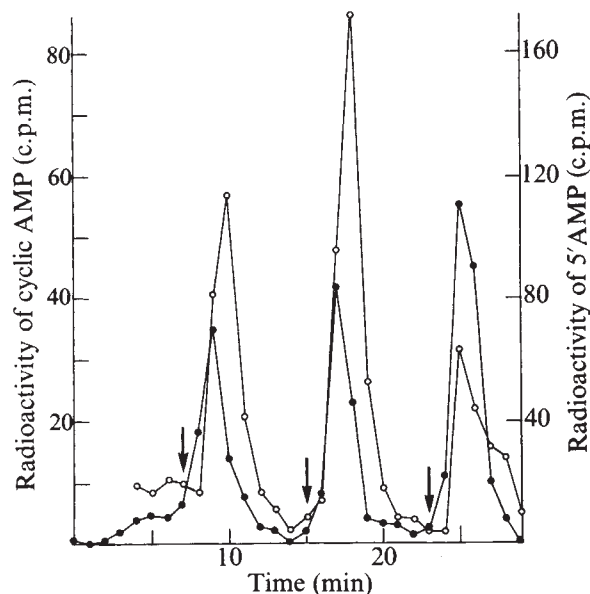


Fig. 3 Cells deposited at 2,500 mm^{-2} , with 3 H-adenine, and swirled at 50 r.p.m. for 14 h until stippled aggregation, then washed. Perfused stationary at 20 °C. 10^{-6} M cyclic AMP added as shown by arrows. Each 1 ml collected added to 0.1 ml 0.1 N formic acid. ●, Radioactivity of cyclic AMP; ○, 5'-AMP after elution from IBAA chromatogram and electrophoresis in 50 mM phosphate at pH 8. Decomposition during a lengthy delay before the last 8 min of the third 5'-AMP peak accounts for the low value of the third 5'-AMP peak.

was indeed a cascade, the secreted agent involved could not have been cyclic AMP. The applied stimulus was, of course, very different from that of normal aggregation, and the continual perfusion, removing both secreted and leaked metabolites, would have tended to interrupt any feedback control loops shortening the response. But conditions were sufficiently normal for the few small aggregations with streams present at the start of the earlier experiments to persist through them. Similar time relationships have recently been found²³ in stirred suspensions of aggregation-competent *D. discoideum* cells. Added cyclic AMP induces two periods of reduced absorbance, the first reaching its minimum after 30 s, the other after 80 s. The slow reaction was thought to be equivalent to the propagation of waves in a cell layer, the fast one to the chemotactic response. There was evidence of a refractory period.

The duration of the transmitted response strongly influences the model of aggregation. An extended mathematical treatment¹⁸ has concluded that the chemotactic signal reaching a cell lasts only 0.2 s, and reversing the sequence of my model⁶, that the cell itself signals 15 s later, and only subsequently begins its oriented movement, which lasts 100 s. The calculated brevity of the received signal, actually a consequence of assuming signal release to be durationless, requires the cell to remain chemotactically refractory throughout this whole period. Recent observations¹² indicate that any refractory period of movement in fact lasts less than 12 s, thus again raising the question of why cells do not reorient to cells signalling behind them, albeit that a lengthier refractory period of secretion can prevent the centripetal transmission of pulses. The answer may lie in the profile and duration of the propagated pulse⁷, and the effect of the cell's own secretion on its responsiveness to other sources.

That cyclic AMP induces secretion of cyclic AMP contrasts with the observation that it inhibits it. Although the latter result was obtained after exposure for many hours to 10^{-4} M cyclic AMP²², this negative feedback may nevertheless be of physiological importance, for aggregates of extremely diverse size are often about equally attractive^{5,24}. It was concluded⁷ that the acrasin output of an aggregate, per cell, is inversely related

to aggregate size, and therefore that secretion can be in some way inhibited, possibly, though not necessarily, by acrasin itself, acting extracellularly or intracellularly.

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Lysis mediated by T cells and restricted by H-2 antigen of target cells infected with vaccinia virus

VARIOUS virus infections lead to the formation of cytotoxic lymphocytes (CL), which are capable of killing virus-infected target cells¹⁻⁴. Specific lysis of target cells infected with ⁵¹Cr-labelled vaccinia virus could be observed when investigating the cell-mediated cytotoxic reaction to vaccinia virus⁵; the CL could be characterised as a T cell. The sensitised lymphocytes from C3H mice could only kill syngeneic L929 cells infected with vaccinia virus, whereas lysis by sensitised lymphocytes derived from DBA/2 mice was restricted to the syngeneic infected mastocytoma P815X2 cells. In the lymphocytic choriomeningitis infection the target cell lysis was shown to be restricted by H-2 antigen⁶. We report here experiments with primary fibroblasts of the mouse strains C3H, DBA/2 and the (C3H×DBA/2)F₁ generation were designed to affirm that the effector phase of virus-specific lysis of target cells mediated by T cells is restricted by H-2 antigen even in the vaccinia virus infection. Further experiments with H-2 alloantisera were performed to indicate the close local relationship between H-2 antigens and viral surface antigens.

Primary embryonic fibroblasts of the strains C3H (H-2^{ka}), DBA/2 (H-2^{db}) and the (C3H×DBA/2)F₁ generation of the two strains (H-2^{ka db}) were prepared, infected with vaccinia virus and used as targets for virus-specific cell mediated cytotoxicity. Lymphocytes were collected from mice sensitised 6 d previously. CL from the strains C3H (H-2^{ka}), DBA/2 (H-2^{db}) and the F₁ (H-2^{ka db}) generation were incubated with each type of the three infected target cells. The CL sensitised to vaccinia virus could lyse only syngeneic infected target cells or the semiallogenic infected cells from the F₁ generation, as was the case with permanent cell lines. Accordingly the hybrid CL from the F₁ generation mice were able to kill each of the infected targets (Table 1).

To investigate the local relationship between virus-induced surface antigens and H-2 antigens we tested

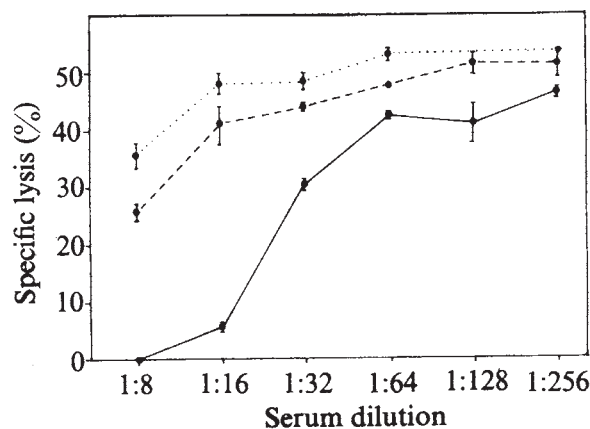


Fig. 1 Blocking effect of H-2 alloantisera on lysis of virus-infected target cells: L929 fibroblasts infected with vaccinia virus. Cytotoxic lymphocytes: spleen cells from C3H mice sensitised to vaccinia virus 6 d previously. Target cells and lymphocytes were incubated together with: —, anti-H-2^{ka} serum; ····, anti-H-2^{db} serum; ----, normal mouse serum.

whether H-2 alloantisera could inhibit the lysis of virus-infected target cells. These sera had no cross reactivity against vaccinia virus. Significant blocking of lysis of infected L929 fibroblasts or mastocytoma P815X2 was observed after incubation of antisera together with CL and infected target cells (Table 2). This effect was restricted by H-2; anti-H-2^{db} sera could inhibit the virus-specific lysis of infected mastocytoma P815X2 cells, but not of infected L929 fibroblasts by CL from DBA/2 mice or C3H mice, respectively. Blocking could be observed up to a serum dilution of 1:256 when titrations of the H-2 antisera were performed (Fig. 1). Infected F₁ cells were used as targets and incubated with sensitised DBA/2 or C3H lymphocytes with addition of normal, anti-H-2^{ka} or anti-H-2^{db} serum to determine whether the blocking was effective on the target cell or on the effector cell level (Table 3a). In this experiment the action of each CL was inhibited by both of the alloantisera, suggesting that the site of blocking is on the target cell. Secondly, CL from the F₁ generation were prepared and their activity was tested against the H-2^{ka} and H-2^{db} target cells with the addition of H-2 alloantisera. The results clearly show that blocking was achieved only when an antibody was added, which was directed against H-2 antigens of the target cells (Table 3b). Thirdly, we preincubated either the target cell (L929 fibroblasts) or the CL (C3H) with the specific alloantiserum for 30 min at 37 °C, washed them twice with fresh medium and performed the test. As shown in Table 4 preincubation of CL was ineffective, whereas preincubation of the infected target cell caused inhibition of virus-specific lysis.

The results support the concept that the action of virus-specific sensitised CL is restricted by the H-2 gene. Our findings in the vaccinia model are consistent with the observations in the LCM system⁶ and similar results were obtained in the ectromelia virus system^{4,13}. This restriction is clearly demonstrated for the effector phase *in vitro* and *in vivo*⁷, but it may also be operative in the sensitisation phase⁸. Zinkernagel and Doherty⁹ pointed out that this restriction does not reflect a physiological interaction with H-2 but rather the immunological surveillance against altered self components.

After addition of H-2 antisera to the *in vitro* test or preincubation of target cells with H-2 antisera significant blocking of target cell lysis can be achieved. This blocking effect is not caused by inhibition of the antigen-specific receptor of the cytotoxic T cell¹⁰⁻¹². This observation indicates a very close topological relationship between the target antigens and H-2 on the cell surface. This target antigen

Table 1 Cytotoxic effect of CL on primary fibroblasts infected with vaccinia virus

Target cell*	Lymphocyte origin	% ⁵¹ Cr release†	% Specific lysis‡
C3H primary fibroblasts infected with vaccinia virus	Normal C3H	26.9±4.9	—
	Immune C3H	41.3±1.6	14.4
	Normal DBA/2	34.6±0.8	—
	Immune DBA/2	33.8±0.3	—
	Normal F ₁ (C3H×DBA/2)	36.6±1.0	—
	Immune F ₁ (C3H×DBA/2)	70.0±0.7	34.4
DBA/2 primary fibroblasts infected with vaccinia virus	Normal C3H	55.4±2.9	—
	Immune C3H	37.0±2.0	—
	Normal DBA/2	39.7±2.0	—
	Immune DBA/2	50.2±2.0	10.5
	Normal F ₁ (C3H×DBA/2)	39.2±4.4	—
	Immune F ₁ (C3H×DBA/2)	64.1±3.4	24.9
(C3H×DBA/2)F ₁ primary fibroblasts infected with vaccinia virus	Normal C3H	51.1±1.3	—
	Immune C3H	62.6±2.1	11.5
	Normal DBA/2	46.5±1.1	—
	Immune DBA/2	59.8±1.8	13.3
	Normal F ₁ (C3H×DBA/2)	30.4±1.2	—
	Immune F ₁ (C3H×DBA/2)	58.8±2.3	28.4

*Primary embryonic fibroblasts from the strains C3H, DBA/2 and the (C3H×DBA/2)F₁ generation were prepared. The second or third passage of these cells was infected with 10 TCID₅₀ per cell of vaccinia virus (strain WR) 3 h before the cytotoxic assay. Production of virus-specific surface antigens was assayed by immunofluorescence. Virus-specific membrane fluorescence was visible after 2 h, reaching 80% stained cells after 20 h. CL were prepared⁶ from spleens from mice infected intraperitoneally 6 d previously with 1 ml 10⁶ TCID₅₀ ml⁻¹ vaccinia virus. The test was performed using a ratio of 100 lymphocytes per target cell⁸. Incubation was performed over a period of 20 h. Spontaneous ⁵¹Cr release from L929 or mastocytoma P815X2 target cells did not usually exceed 30%. Primary fibroblasts were much more sensitive to vaccinia virus infection and often showed high spontaneous release of the label; the addition of normal lymphocytes had a feeding effect and reduced spontaneous cytolysis.

†Each value represents mean ± s.e.m. of *n* = 5 wells, significance of specific lysis was affirmed by Students' *t* test (*P* < 0.01).

‡Specific lysis was calculated by subtracting ⁵¹Cr release in presence of normal lymphocytes from ⁵¹Cr release in presence of sensitised cells. Spontaneous release of radioactivity from non-infected fibroblasts did not exceed 35%. CL had no unspecific activities on non-infected cells. Spontaneous release from infected fibroblasts was strain-dependent and varied between 30–50%.

may be either a specifically altered H-2 antigen (altered self), different from viral surface antigens, or it may be a non-specifically altered self plus viral surface antigen⁹ and has an unknown topological relationship with both antigens. The blocking activity of H-2 antibodies favours both concepts. There can only be slight antigenic modifications otherwise the H-2 antiserum would no longer bind to the

antigen. The blocking of the cytolytic reaction after addition of heterologous vaccinia antiserum⁹ without cross reactivity to H-2 antigens seems to be more in favour of the second possibility. But it is also not at odds with the first assumption, as steric hindrance of the target antigen cannot be excluded. In preliminary experiments we have found different patterns of H-2 distribution on the surface of

Table 2 Effect of H-2 alloantisera on lysis of virus-infected target cells

Target cell	Lymphocytes	Serum	% ⁵¹ Cr release	% Specific* lysis	% Blocking
Mastocytoma cells infected with vaccinia virus	—	Normal	29.2±0.2	—	—
	Immune DBA/2	Normal	46.3±0.7	17.1	—
	—	Anti-H-2 ^{dd}	45.4±0.6	—	—
	Immune DBA/2	Anti-H-2 ^{dd}	46.5±1.6	0.9	16.2
	—	Anti-H-2 ^{kk}	36.0±1.0	—	—
	Immune DBA/2	Anti-H-2 ^{kk}	55.0±1.0	19.0	—
L929 cells infected with vaccinia virus	Normal DBA/2	—	40.8±0.8	—	—
	Immune DBA/2	—	69.9±1.1	29.1	—
	—	Normal	17.7±0.8	—	—
	Immune C3H	Normal	43.7±1.2	26.0	—
	—	Anti-H-2 ^{dd}	16.6±1.7	—	—
	Immune C3H	Anti-H-2 ^{dd}	52.3±2.1	25.7	—
	—	Anti-H-2 ^{kk}	18.2±1.0	—	—
	Immune C3H	Anti-H-2 ^{kk}	16.7±0.8	—	26.0
	Normal C3H	—	18.0±0.4	—	—
	Immune C3H	—	68.3±5.5	50.3	—

*Specific lysis was calculated by subtraction of lysis in presence of serum from lysis in presence of serum and lymphocytes.

†Blocking effect of H-2 alloantisera was calculated by subtraction of lysis in presence of H-2 alloantisera and lymphocytes from lysis in presence of normal serum and lymphocytes.

H-2 alloantisera were obtained from Dr J. G. Ray, Transplantation and Immunology Branch, NIAID, NIH, Bethesda, Maryland. We used as anti-H-2^{kk} serum D-3b, raised in the recipient-donor strains (C3H-H-2^c × 129) anti-C3H; genotypes were (H-2^{d/k} × H-2^{b/b}) anti-H-2^{kk}. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52, and also haemagglutinating for specificity 3. As anti-H-2^{dd} serum we used D-31, raised in recipient-donor strains (B10 × A) anti-B10.D2; genotypes were: (H-2^{b/b} × H-2^{k/d}) anti-H-2^{dd}; anti-H-2 specificities were: 31, 34. Details of the raising and testing of these sera are described in the NIH catalogue¹⁴.

Table 3 Site of blocking mediated by H-2 alloantisera

Target cell	Lymphocytes	Serum	% ⁵¹ Cr release	% Specific lysis	% Blocking
(a) (C3H × DNA/2)F ₁ primary fibroblasts infected with vaccinia virus	—	Normal	47.0 ± 3.0	—	—
	Immune DNA/2	Normal	59.8 ± 3.4	12.8	—
	Immune C3H	Normal	60.4 ± 1.9	13.4	—
	—	Anti-H-2 ^{dd}	43.2 ± 1.7	—	—
	Immune DBA/2	Anti-H-2 ^{dd}	44.8 ± 1.7	1.6	11.2
	Immune C3H	Anti-H-2 ^{dd}	44.2 ± 1.0	1.6	12.4
	—	Anti-H-2 ^{kk}	50.2 ± 1.9	—	—
	Immune DBA/2	Anti-H-2 ^{kk}	46.3 ± 2.0	—	12.8
	Immune C3H	Anti-H-2 ^{kk}	41.1 ± 1.5	—	13.4
	—	—	—	—	—
F ₁ primary fibroblasts infected with vaccinia virus	Normal DBA/2	—	46.8 ± 0.9	—	—
	Immune DBA/2	—	59.9 ± 1.8	13.3	—
Uninfected F ₁ primary fibroblasts	Immune DBA/2	—	47.0 ± 2.0	—	—
	Immune C3H	—	40.7 ± 1.5	—	—
(b) L929 cells infected with vaccinia virus	—	Normal	26.6 ± 1.0	—	—
	Immune (DBA/2 × C3H)F ₁	Normal	67.6 ± 2.6	40.9	—
	—	Anti-H-2 ^{kk}	36.9 ± 3.1	—	—
	Immune (DBA/2 × C3H)F ₁	Anti-H-2 ^{kk}	31.4 ± 1.1	—	40.9
	—	Anti-H-2 ^{dd}	50.5 ± 3.6	—	—
	Immune (DBA/2 × C3H)F ₁	Anti-H-2 ^{dd}	67.6 ± 2.0	41.0	—
	Normal (C3H × DBA/2)F ₁	—	38.5 ± 3.1	—	—
	Immune (C3H × DBA/2)F ₁	—	62.0 ± 2.4	23.6	—
	Uninfected L929 cells	—	18.9 ± 1.8	—	—
	—	—	—	—	—

Table 4 Effect of preincubating CL or targets with H-2 alloantisera on target lysis cell

Target cell	Lymphocytes	Serum	% ⁵¹ Cr release	% Specific lysis	% Blocking
L929 cells infected with vaccinia virus	—	Normal	17.9 ± 0.8	—	—
	Immune C3H	Normal	33.8 ± 2.4	15.9	—
	—	Anti-H-2 ^{kk}	18.6 ± 1.9	—	—
	Immune C3H	Anti-H-2 ^{kk}	9.7 ± 1.0	—	15.9
	Immune* C3H	Normal	29.4 ± 0.6	11.6	—
	Immune* C3H	Anti-H-2 ^{kk}	13.1 ± 1.3	—	11.6
	Immune† C3H	Normal	28.8 ± 2.1	10.9	—
	Immune† C3H	Anti-H-2 ^{kk}	26.6 ± 1.5	8.0	2.9
	Normal C3H	—	22.5 ± 2.0	—	—
	Immune C3H	—	39.4 ± 2.0	16.9	—
L929 cells infected with vaccinia virus	Immune C3H	—	19.9 ± 1.2	—	—
Uninfected L929 cells	Immune C3H	—	—	—	—

*Target cells preincubated with serum.

†Lymphocytes preincubated with serum.

infected and non-infected cells by immunofluorescence. In absorption studies vaccinia-infected cells had a reduced capacity to absorb H-2 antisera. Secondly, preincubation of the infected cells with H-2 antisera reduced the vaccinia specific fluorescence. This again suggests a close topological relationship between both antigens.

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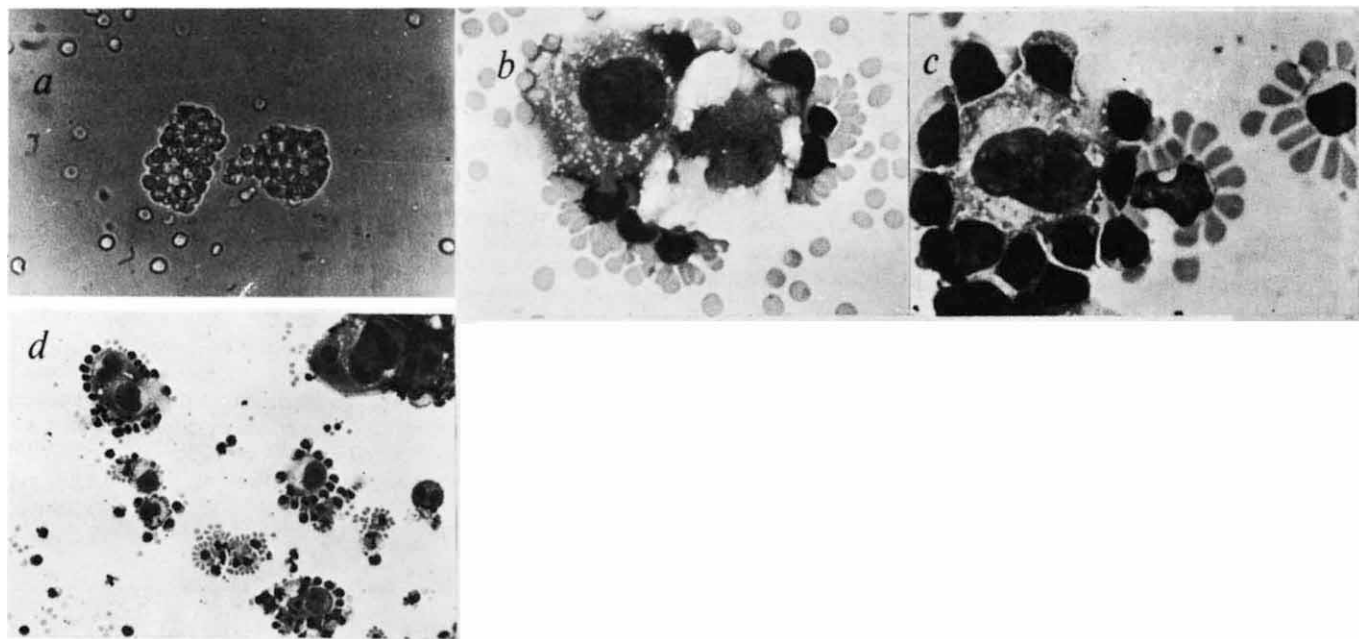
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Measles virus receptor on human T lymphocytes

ONE of the factors which may determine whether or not a cell is susceptible to a particular virus is the presence in the cell membrane of a receptor or a binding site for the virus¹. It has been demonstrated that certain viruses, including herpes and myxoviruses, can infect and multiply within lymphocytes^{2,3}. Following von Pirquet's original observation⁴ it is now well recognised that acute measles infection is associated with depression of cellular (T lymphocyte-dependent) immunity.

More recently, striking cytopathic changes have been described in the thymus of children during and shortly after measles⁵. Furthermore, blood lymphocytes from measles patients form giant cells when cultured *in vitro*⁶. In a cytotoxicity assay devised to study cellular immunity to measles *in vitro* (unpublished), we observed that lymphocytes seemed to cluster around measles-infected fibroblasts in far greater numbers than would be expected if only specifically measles-reactive lymphocytes were involved. We therefore decided to investigate further the nature of this clustering phenomenon. It was found that the majority of human T lymphocytes have surface binding sites for measles virus and, on binding, were cytotoxic to measles-infected cells regardless of the donor's previous experience of measles infection.



A cell line carrying measles virus (Mc-Lu 106) (ref. 7) was used and uninfected control cells (Lu 106) of the same origin.

Binding of measles virus to human lymphocytes was demonstrated by a rosetting technique. Monolayers of infected and uninfected cells were trypsinised and incubated at 37 °C in HEPES buffered medium (HBM) with 5% FCS for a minimum of 2 h. The cells were then washed twice and resuspended in serum-free HBM to a concentration of 10^6 viable cells ml^{-1} . Lymphocytes were obtained from human blood by the Hypaque-Ficoll method⁸, washed twice and suspended in serum-free HBM to a concentration of 5×10^5 – 5×10^6 ml^{-1} . Equal volumes of lymphocytes and measles carrier cells (or Lu 106 control cells) were mixed at ratios from 5:1 to 50:1 in round-bottomed test tubes (LP₂, Luckham), centrifuged (300g) for 7 min at room temperature and gently resuspended. The cells were examined for rosette formation (LM rosettes) in a haemocytometer, and Giemsa stained cytocentrifuge preparations. Measles carrier cells with three or more lymphocytes attached were counted as rosettes. In optimal conditions 70–90% of the measles-infected carrier cells formed rosettes with human lymphocytes (Fig. 1a). Similar proportions of the measles carrier cells showed strong measles-specific membrane fluorescence. Uninfected control cells (Lu 106) showing no measles-specific fluorescence, did not form rosettes. The LM rosettes were also formed by lymphocytes from individuals who had no history of measles as confirmed by absence of measles antibodies. At a low lymphocyte-carrier cell ratio (5:1), 60–70% of human blood lymphocytes were bound by the measles-infected cells. Lymphocytes from several animal species were tested for measles virus receptor. Substantial binding was

only observed with monkey, and to a much lesser extent with ox, dog and pig lymphocytes. Other species tested showed minimal or no binding.

Preincubation of the carrier cells with measles antibodies consistently inhibited rosette formation (Table 1). Attempts to block the binding with measles virus were unsuccessful because the lymphocytes were agglutinated by the virus. Trypsinisation of either cell type prevented binding but treatment with neuraminidase and fetuin did not inhibit the rosette formation.

The measles-reactive lymphocytes were further characterised by incubating LM rosettes with sheep erythrocytes (E) and IgG-sensitised ox red cells (EA) (ref. 9). Equal volumes of LM rosettes and 1% red cell suspensions were mixed in LP₂ tubes, and centrifuged for 7 min (300g) at room temperature

and the rosettes examined as before. Most of the carrier cell-attached lymphocytes were firmly surrounded by the sheep red cells (Fig. 1b). In contrast, EA did not adhere to bound lymphocytes but formed rosettes with the non-adherent lymphocytes (Fig. 1c). Experiments in which the carrier cells were rosetted with B-lymphocyte enriched population, containing approximately 20% E and 54% EA-binding cells, showed a reduced number of LM rosettes, and those formed had few lymphocytes attached. Again, the attached lymphocytes failed to bind EA, whereas the majority of the free lymphocytes did (Fig. 1d). Using T-lymphocyte enriched population (less than 5% EA-binding lymphocytes), almost all the lymphocytes were bound and formed strong LM rosettes with the carrier cells. Polyvalent fluorescein-conjugated rabbit anti-human immunoglobulin serum (Hoechst) was used in a direct assay for membrane immunoglobulins. LM rosettes were formed as

Table 1 Inhibition of LM rosettes by pretreatment of carrier cells with measles antibodies

Carrier cells preincubated in	Percentage carrier cells binding lymphocytes*		
	Exp. 1	Exp. 2	Exp. 3
Measles-negative serum	23.3	82.6	87.5
Measles-positive serum	14.0	14.6	61.8

Suspension of carrier cells incubated for 1 h at 37 °C in 1:50 dilution of sera with measles antibody titres of <1:4 and 1:2,048 respectively for haemagglutination inhibition.

*Lymphocyte:carrier cell ratio 10:1.

described above (lymphocyte-carrier cell ratio 10:1) and suspended in an appropriate dilution (1:5 of this antiserum. After 30 min incubation on ice and 3 washes at 4 °C, bound and unbound lymphocytes were examined for membrane fluorescence under the ultraviolet microscope. In three experiments 1.8–4.0% of the carrier cell attached lymphocytes reacted with this antiserum compared with 29–39% of the unbound lymphocytes.

After binding, the lymphocytes were observed to kill measles-infected target cells *in vitro* and this was readily demonstrated by the Trypan blue method. In a chromium release assay, 5×10^4 carrier cells or uninfected control cells were put into round bottomed test tubes. Human lymphocytes were added to give duplicates of the appropriate lymphocyte target cell ratios (2, 5:1 to 40:1). The final volume of each culture was 1 ml of bicarbonate-buffered 199 medium containing 10% FCS. The cells were centrifuged (300g) for 7 min and the pellets gently resuspended before incubation at 37 °C in 5% CO₂ and 95% air. After 1–24 h the tubes were centrifuged and 0.5 ml of medium aspirated for gamma counting.

In this assay, measles-dependent cytotoxic effect could be detected within 2 h, increasing in a linear fashion over 18–24 h. Using 18 h incubation, maximal cytotoxicity was usually observed at a lymphocyte:target cell ratio between 20 and 40. At ratios above 10, however, the measles-dependent effect was sometimes reduced as a result of chromium release from the uninfected control cells. Inhibition of ⁵¹Cr release from the measles carrier cells was also occasionally seen at lymphocyte:target cell ratios greater than 20.

Table 2 Toxicity of measles-negative and measles-positive lymphocytes to measles-infected cells

Lymphocyte-target cell ratio	Percentage measles-dependent cytotoxicity*					
	Measles-positive† subjects			Measles-negative‡ subjects		
	Mean	Range	n	Mean	Range	n
2.5:1	9.3	0–12.8	3	2.2	0–8.2	4
5:1	16.2	2–38.6	5	8.2	1.3–19.5	5
10:1	31.0	6.0–48.2	6	16.3	8.1–21.5	5
20:1	42.8	13.7–75.5	9	22.5	3.8–79.0	10
40:1	53.0	9.1–86.7	5	23.1	6.4–77.7	7

*Obtained by subtracting the percentage cytotoxicity observed in uninfected control cells from that of measles-infected carrier cells.

†Ten subjects tested.

‡Nine subjects tested.

It is evident from Table 2 that lymphocytes from both measles-negative and measles-positive subjects were toxic to measles-infected cells, indicating that the killing did not require the presence of measles-specific lymphocyte clones. Although both groups show considerable scatter, the mean cytotoxicity of lymphocytes from measles-positive subjects was higher than that from those of measles-negative subjects at all lymphocyte:target cell ratios.

We have shown that the majority of human T lymphocytes, and few, if any B lymphocytes, have surface binding sites for measles virus. The reverse has been reported for Epstein-Barr virus, which binds to B but not to T lymphocytes¹⁰. Our findings also suggest that cell-associated measles virus may activate human lymphocytes nonspecifically.

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Effect of concanavalin A and its derivative on the potassium compartmentation of Ehrlich ascites tumour cells

CONCAVALIN A (con A) has become an interesting probe for the study of dynamic structures of the cell surface^{1–3}, and of mechanisms of metabolic stimulation such as lymphoid cell transformation^{4,5}, polymorphonuclear leukocyte respiration⁶, and of selective killing of the transformed cells⁷. The critical initial event in these phenomena is the binding of con A to specific receptor sites on the cell surface. One of the most important problems is to determine how interactions of surface receptors with this ligand are linked to the intracellular metabolism. We report here the effect of con A and its succinylated derivative (succinyl con A) on cell agglutination and the K⁺-compartmentation of Ehrlich ascites tumour cells (EATC).

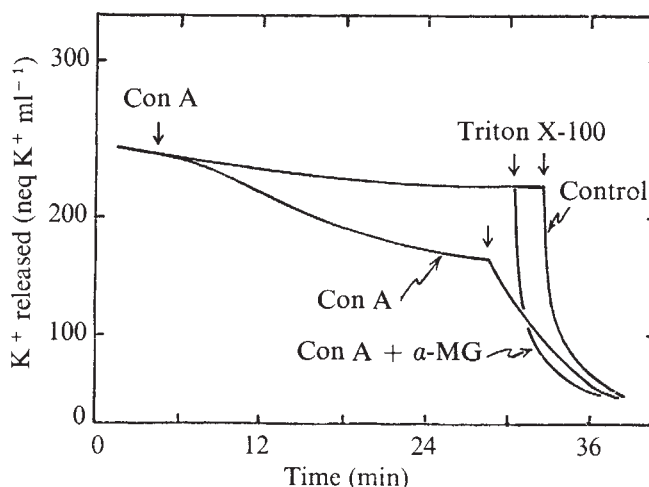


Fig. 1 Effect of con A on the K⁺-compartmentation of Ehrlich ascites tumour cells. Cells (2×10^6 ml⁻¹) were incubated with con A (50 µg ml⁻¹) at 25 °C in the presence or absence of 10 mM α-MG. At the end of incubation, Triton X-100 (0.1%) was added to release all K⁺ remaining within the cells. Other conditions were described in the text.

EATC were collected from ascites fluid of ddN mice 7 d after intraperitoneal transplantation. Cells were washed 3 times with 0.34 M mannitol containing 10 mM Tris-HCl, pH 7.4. Con A was purified from jack bean meal by the method of Agrawal and Goldstein⁸. Succinyl con A, with the same affinity constant for α-methyl-D-glucoside (α-MG) as native con A, was prepared by the method of Gunther *et al.*⁹, and further purified by affinity chromatography of Sephadex G-100. They were dissolved in 0.35 M mannitol containing 1 mM CaCl₂ and

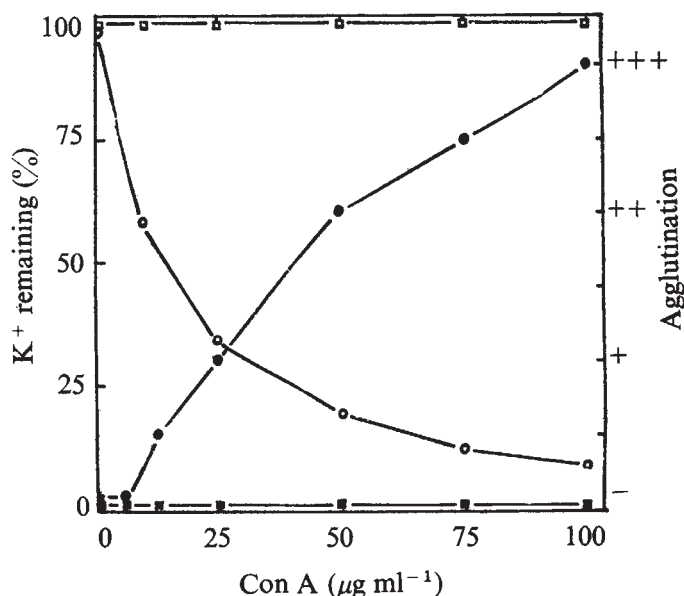


Fig. 2 Effect of con A and succinyl con A on agglutination and K⁺-compartmentation of Ehrlich ascites tumour cells. Cells (3.4×10^6 ml⁻¹) were incubated with varying concentrations of con A or succinyl con A at 37 °C for 20 min. K⁺ remaining within the cells was calculated after releasing it from the cells by Triton X-100 (0.1%). Agglutination induced by con A (●), or by succinyl con A (□); K⁺ remaining within the cells, con A (○), succinyl con A (□).

1 mM MnCl₂. All reagents used were of reagent grade. Cell agglutination was scored as described previously¹⁰.

Release of K⁺ from EATC was measured in the same buffer used for cell washing by K⁺-electrode (Electronic Instrument Ltd, Model C-KN33C) connected to a pH-meter (Toa Electronic Ltd, Model HM-20B).

Figure 1 shows the effect of con A on the K⁺-compartmentation of EATC. There is a rapid induction of K⁺-release from EATC after addition of con A, in agreement with the data reported by Aull and Nachbar¹². K⁺-release induced by con A is completely inhibited by α-MG, a haptenic inhibitor of this lectin. The extent of K⁺-release from EATC increased progressively with increased concentration of con A (Fig. 2). Cell agglutination of EATC was also induced in these conditions.

Succinylated derivative of con A, however, induced neither cell agglutination nor K⁺-release from EATC in the same conditions. Quite a close relationship between the two phenomena was observed as a function of con A concentration. Both of these phenomena induced by con A were temperature dependent, and observed at temperatures above 15 °C (Fig. 3). But, succinyl con A lacks such activity at any temperature tested.

Our results suggest that the binding of tetrameric con A to the surface glycoprotein receptors of EATC leads to a change in the properties of the surface membrane, which results in an induction of cell agglutination and K⁺-release from the cell at temperatures above 15 °C. Edelman suggested that tetrameric con A, and not dimeric succinyl con A, may function to induce cross-linking of surface glycoprotein receptors into micro-patches⁹, which is inhibited at low temperatures (<10 °C) because of decreased fluidity of the surface membrane¹¹.

The same mechanism of con A action that underlies glycoprotein receptor cross-linking may be operating in the changes in K⁺-compartmentation of EATC. The next objective of our study is to observe how such a change in K⁺-compartmentation induced by con A affects cellular metabolism.

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Specific alterations of olfactory function in humans with temporal lobe lesions

SUBSTANTIAL anatomical and electrophysiological evidence suggests that human temporal lobe structures are involved in the processing of odour perception. The temporal lobe has both afferent and efferent connections with the olfactory bulb^{1,2}, the major synaptic junction for primary fibres from the olfactory receptors. Olfactory auras accompany epileptic seizures of temporal lobe origin³, and electrical stimulation of the uncus and amygdala in conscious patients elicits odour sensations⁴. There are, however, no known quantitative studies of olfactory function in patients with surgically verified temporal lobe lesions. The present findings demonstrate that removal of the temporal lobe increases odour detection thresholds (ODT), whereas odour recognition thresholds (ORT) remain similar to those of subjects without brain damage.

Odour thresholds were determined in patients with surgically defined lesions of the temporal lobe. These subjects had undergone unilateral excision of the anterior segment of the temporal lobe to relieve otherwise intractable epileptic seizures⁵. The lesions extended 5 to 7 cm posterior from the anterior projection of the lobe. This area included the uncus, amygdala

Fig. 3 Effect of temperature on agglutination and K⁺-compartmentation. Concentration of con A or succinyl con A used was 50 μg ml⁻¹. Other conditions were the same as Fig. 2. Agglutination induced by con A (●), or by succinyl con A (■); K⁺ remaining within the cells, Con A (○), succinyl con A (□).

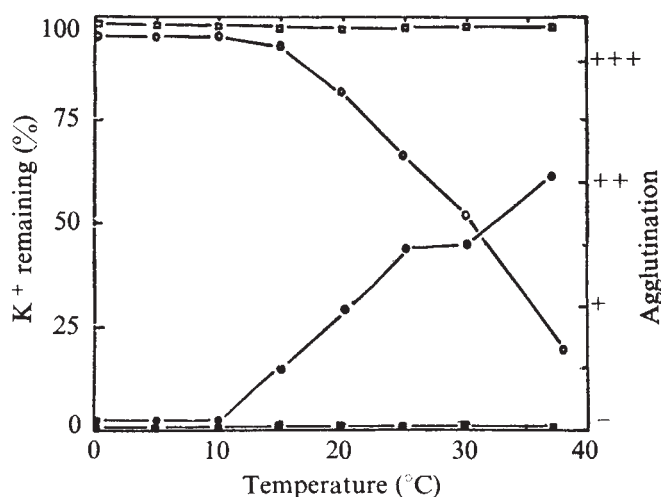


Table 1 Effects of temporal lobe ablation on ODT and ORT

	Pyridine		— log molar concentration of odour (mean $\pm$ s.e.m.)		Phenethyl alcohol	
	ODT	ORT	ODT	ORT	ODT	ORT
Temporal lobectomy	3.58 $\pm$ 0.08*	3.08 $\pm$ 0.07	4.58 $\pm$ 0.11*	3.91 $\pm$ 0.08	5.00 $\pm$ 0.13*	3.72 $\pm$ 0.09
Control	5.60 $\pm$ 0.12	3.50 $\pm$ 0.09	7.50 $\pm$ 0.13	4.20 $\pm$ 0.11	9.90 $\pm$ 0.16	5.00 $\pm$ 0.13

* Significantly different from control at $P < 0.01$.

The forced-choice sniff bottle technique with three stimuli⁸ was used to determine ODT and ORT in the test groups. In brief, three 125 ml Erlenmeyer flasks with 18 ml solution were presented in random order to each subject who was then instructed to identify the bottle containing the test odour. Odour determination by random choice is not significant ($P = 0.0004$). In each trial, one flask contained the test odour, pyridine ($\text{CH}(\text{CH}_3)_2\text{N}$; Mallinckrodt, St Louis, Missouri), phenethyl alcohol ($\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{OH}$; Matheson, Coleman and Bell, Los Angeles, California) or pentyl acetate ($\text{CH}_3\text{CO}_2(\text{CH}_2)_4\text{CH}_3$; Matheson, Coleman and Bell) while the remaining flasks contained the solvent, Millipore-filtered and distilled water. Concentrations of pyridine from 10 M to 10^{-12} M were verified by spectrophotometric measurements at 260 nm to check the accuracy of our dilution technique. As the concentration of the test odour increased in successive trials, a transition occurred where the response of the subject changed from random selection to the correct choice with consistency. Re-testing was carried out about this point. The ODT was defined as the lowest odour concentration at which a subject gave two successive correct responses while giving two consecutive incorrect responses at the next lower concentration. The ORT was determined as follows. Before any trials were given, the subject was shown a short list of adjectives describing four odours. On each of the test trials, the subject was asked to identify its principal characteristics from the list. The ORT was defined as the lowest odour concentration level at which the subject could correctly identify the odour characteristics. Right and left lobectomy patients are grouped together as neither ODT nor ORT of patients with excisions in the right temporal lobe differed significantly from those of left temporal lobectomy patients ($P > 0.05$), determined by analyses with the t statistics¹⁶. The ability of both lobes of the brain to recognise odours is supported by studies in split-brain patients¹⁶.

and portions of the hippocampus, hippocampal gyrus and the fusiform gyrus. All patients were examined at least one year after surgery to avoid possible postoperative effects of oedema on test results. Odour thresholds of 12 right-handed surgical subjects, seven with right and five with left temporal lobectomies, were compared with those of ten control subjects who had no history of damage in the central nervous system.

ODT and ORT levels determined for surgical and control subjects are presented in Table 1. Temporal lobectomy patients had significantly higher ODT levels than controls for all odours tested at $P < 0.01$. Conversely, ORTs of the lobectomy patients were not significantly different from the ORT levels of subjects in the control group. The results demonstrate further that the ODT and ORT for each odour were more often at the same concentration level for subjects in the surgical group than were those in the control group ($P < 0.001$, χ^2 test).

These data indicate that temporal lobe lesions impair the ability of human subjects to detect, but not to recognise odours at threshold levels. Whereas control subjects can detect an odour at a concentration which is too weak for identification of its characteristics, temporal lobectomy patients can detect an odour only at a concentration level which is strong enough for recognition. This ODT impairment does not seem to result from a deficiency in attention, as intelligence (measured on the Wechsler Scale⁷) and attention (measured by digit span) show no correlation with ODT. In view of these results, it is noteworthy that most of the lobectomy patients rated their olfactory ability as average in their subjective judgment.

Surgical removal of human brain tissue from several neural regions other than the temporal lobe does not elicit consistent alterations in olfactory sensitivity⁸. Tumours in and around the frontal and possibly temporal lobe areas, however, have been reported to induce increments in olfactory thresholds⁹. In studies of the effects of bilateral amygdaloid lesions, transient changes in several olfactory judgments were reported¹⁰. In-depth EEG evoked responses in the amygdala^{10,11} and widespread changes in the unit activity of temporal lobe structures¹² have also been observed in response to olfactory stimuli.

Collectively, these data implicate the human temporal lobe in olfactory information processes, but the nature of that role remains unknown. The potential influences of the ongoing θ rhythm¹³ and the other efferent inputs¹⁴ on the transmission of olfactory information to neural areas involved in odour perception should be investigated further. Nevertheless, the present findings indicate that increments in ODT accompany lesions of temporal lobe structures.

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Muscarinic response to acetylcholine in neuroblastoma $\times$ glioma hybrid cells

THE complexity of the central nervous system of vertebrates makes it difficult to identify the cell type responsible for the biochemical or pharmacological effects of drugs observed in the whole tissue. This difficulty does not exist for clonal cell lines derived from tumours of the nervous system. Such lines derived from mouse neuroblastoma C1300 show a number of differentiated functions of neurones¹. Many of these properties are more strongly expressed in clonal lines of hybrid cells obtained by fusion of mouse neuroblastoma and rat glioma cells (refs 2–4 and B.H., unpublished). In suitable conditions, these undifferentiated cells differentiate by extending long

processes³⁻⁵, which contain structures resembling synaptic vesicles⁶. In addition, their membranes become excitable by an electric current (refs 3 and 4 and B.H., unpublished) or by neurotransmitters (refs 3, 4 and 7, and B.H., unpublished). On exposure to prostaglandin E₁ (PGE₁) hybrid cells strongly increase their intracellular levels of cyclic AMP⁸. This effect of PGE₁ is antagonised by α adrenergic agonists⁷ and morphine^{7,9}.

Here we report that the increase in the intracellular concentration of cyclic AMP evoked by PGE₁ is also suppressed, if cholinergic agonists like acetylcholine (ACh) or carbamylcholine are present. In neuroblastoma $\times$ glioma hybrid cells the effect of the cholinergic agonist is prevented by the blocker of muscarinic receptors, atropine, but not by the inhibitors of nicotinic receptors, D-tubocurarine and α bungarotoxin. Analogous results were obtained with mouse neuroblastoma cells (J.T., unpublished). The neuroblastoma $\times$ glioma hybrid line 108CC15 (refs 3 and 4, and B.H., unpublished) and 108CC25 (ref. 7 and R. Heumann, unpublished) are used, since these are found to be the most responsive to PGE₁.

Hybrid cells were cultured in plastic dishes (Greiner, 8.5 cm in diameter)^{8,10}, and incubated for 10 min at 37 °C, at varying concentrations of cholinergic agonists and antagonists in the absence or presence of 3 $\mu\text{mol l}^{-1}$ PGE₁, as described previously⁹. The compounds, dissolved in Dulbecco's modified Eagle's medium, were added (50 μl) to 5 ml incubation medium. After the incubation, the cells were assayed for cyclic AMP^{10,11}. Analysis of the incubation medium for cyclic AMP that had been released from the cells proved to be unnecessary⁹. The data were referred to cellular protein¹⁰.

The more than 60-fold elevation of the level of cyclic AMP by PGE₁ in the hybrid cells is progressively prevented by increasing concentrations of ACh (Fig. 1a). The ACh concentration at which 50% inhibition takes place (IC₅₀) is 0.1 $\mu\text{mol l}^{-1}$. No obvious inhibition by ACh was observed when the cells were incubated in the absence of PGE₁ (Fig. 1b). To classify the cholinergic receptor mediating the effect of ACh, hybrid cells were incubated in the presence of constant concentrations of PGE₁, ACh, and eserine, and varying concentrations of blockers of ACh receptors. Whereas the muscarinic antagonist atropine¹² potently blocked (IC₅₀ 10 nmol l^{-1}) the inhibitory action of ACh (Fig. 2a), only a very slight effect was observed in the presence of the nicotinic antagonist D-tubocurarine¹² (Fig. 2d). The snake venom α bungarotoxin (pure toxin, Miami Serpentarium Laboratories), a potent inhibitor of nicotinic action of ACh¹³, has no effect when added together with ACh (Fig. 2c), and causes only a very slight effect, when added 30 min before incubation with the other drugs (Fig. 2b). Even at concentrations up to 0.1 mmol l^{-1} no effect of the toxin could be detected (data not shown). The experiment clearly demonstrates that the ACh receptors of the undifferentiated hybrid cells are of the muscarinic type. Electrophysiological experiments on differentiated hybrid cells had demonstrated ACh receptors which were equally sensitive to atropine and D-tubocurarine and which were blocked by α bungarotoxin (refs 3 and 4, and B.H., unpublished). Experiments are under way to elucidate the nature of this change in receptor specificity.

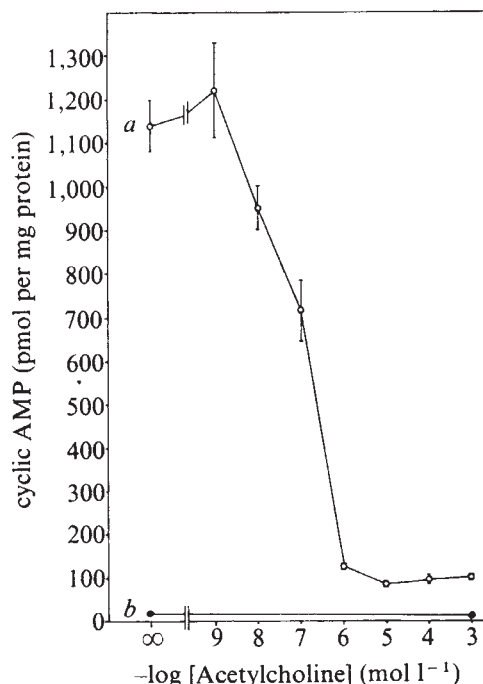


Fig. 1 Inhibition by ACh of the stimulation by PGE₁ of cyclic AMP formation in neuroblastoma $\times$ glioma hybrid clone 108CC15. At varying concentrations of ACh, the cells were incubated in the presence or absence of 3 $\mu\text{mol l}^{-1}$ PGE₁. To prevent breakdown of ACh, the acetylcholinesterase inhibitor eserine (0.1 mmol l^{-1}) was included in all incubations. Subsequently, the cyclic AMP formed was measured (s.d. $\pm$ mean of three parallel incubations except (b), obtained from single incubations), 2.4×10^8 viable cells per plate, passage number 12, viability (exclusion of nigrosin) 96%. The value in the absence of special additions was 16 pmol per mg protein. a, Cyclic AMP in the presence of PGE₁; b, cyclic AMP in the absence of PGE₁.

Certain clones of neuroblastoma C1300 show binding constants for α bungarotoxin of about 10 $\mu\text{mol l}^{-1}$. Muscarinic and nicotinic antagonists are nearly equally effective in preventing the toxin binding¹⁴. This observation is comparable with that on the differentiated hybrid cells, but not with that on the undifferentiated cells described here.

As with ACh, its analogue carbamylcholine also prevents the increase of the intracellular concentration of cyclic AMP observed in the presence of PGE₁ (Table 1). Carbamylcholine also counteracts the elevation of the level of cyclic AMP caused by isobutylmethylxanthine (IBMX), an inhibitor of cyclic nucleotide phosphodiesterase^{15,16}. This result suggests that the effect of the cholinergic agonists consists in lowering adenyl cyclase, rather than increasing phosphodiesterase activity. Acetylcholine increases the levels of cyclic GMP in the hybrid cells (R. Gullis, J.T. and B.H., unpublished). Therefore, the prevention by cholinergic agonists of the increase in cyclic AMP concentration in the presence of PGE₁

Table 1 Effect of carbamylcholine on the levels of cyclic AMP in hybrid cells treated with PGE₁ or IBMX

Addition	Hybrid line	
	108CC15	108CC25
None	10	16
PGE ₁ (3 $\mu\text{mol l}^{-1}$)	811 $\pm$ 109	116 $\pm$ 16
Carbamylcholine (0.1 mmol l^{-1})	12 $\pm$ 1	11 $\pm$ 3
PGE ₁ (3 $\mu\text{mol l}^{-1}$) + carbamylcholine (0.1 $\mu\text{mol l}^{-1}$)	168 $\pm$ 22	60 $\pm$ 3
IBMX (0.5 mmol l^{-1})	41	42
IBMX (0.5 mmol l^{-1}) + carbamylcholine (0.1 mmol l^{-1})	24	23 $\pm$ 1

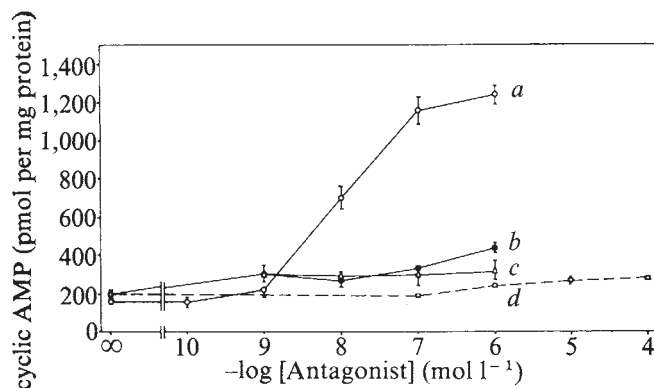


Fig. 2 Effect of cholinergic receptor blockers on the inhibition by ACh of the formation of cyclic AMP stimulated by PGE₁ in neuroblastoma × glioma hybrid cells. Besides PGE₁ all incubations contained 10 $\mu\text{mol l}^{-1}$ ACh, eserine and varying concentrations of cholinergic antagonists. *a*, Obtained in an experiment separate from that which gave rise to the other curves. Cells per plate were 2.6×10^6 and 1.1×10^6 , passage number 14 and 13, viabilities 91.0% and 97.7% for the cells of curves (*a*) and (*b-d*), respectively. Control values: *a*, eserine alone 17, ACh + eserine 10, atropine ($1 \mu\text{mol l}^{-1}$) + eserine 22, atropine + eserine + ACh 18, PGE₁ + eserine 1,100, PGE₁ + eserine + atropine 1,030; *b* and *c*, no additions or ACh alone 10, PGE₁ alone 1,200, PGE₁ + eserine 1,600. *a*, Atropine; *b*, α bungarotoxin, added 30 min before other drugs; *c*, α bungarotoxin added simultaneously with the other drugs; *d*, D-tubocurarine. Toxicity of α bungarotoxin was verified by the death of five mice injected with 4 μg of the drug.

is probably mediated by cyclic GMP. The question of whether cyclic GMP causes a reduction in adenylyl cyclase activity by decreasing the number of receptor sites occupied by PGE₁, or by a more direct action on the enzyme, is under investigation.

In homogenates of a clone of mouse neuroblastoma C1300, ACh is reported to stimulate adenylyl cyclase activity¹⁷. The effect seems to be counteracted equally well by muscarinic and nicotinic cholinergic antagonists at the high concentration of 0.1 mmol l⁻¹. It was concluded¹⁷ that these cells contain both muscarinic and nicotinic cholinergic receptors linked to adenylyl cyclase. Data showing additive effects of both types of antagonists and curves demonstrating the inhibitory effects as functions of the concentrations of the antagonists, were not given. Therefore, the above conclusion¹⁷ seems to be premature. Our results are in agreement with findings that stimulation of muscarinic cholinergic receptors in heart¹⁸, brain¹⁹ and lung²⁰ results in elevated levels of cyclic GMP rather than cyclic AMP. Relationships between hormones elevating cellular levels of cyclic AMP and others antagonising this effect are known for other systems. Somatostatin²¹, noradrenaline²² and insulin²³⁻²⁵ have been shown to counteract the increased levels of cyclic AMP in pituitary glands²¹, platelets²² and fat cells²³⁻²⁵ produced by PGE₂ (ref. 21), PGE₁ (ref. 22) and adrenaline²³⁻²⁵, respectively.

Our results suggest the presence of muscarinic cholinergic receptors in the hybrid cells. These cells may therefore be useful in detailed studies of the molecular properties of muscarinic receptors. Second, in hybrid cells useful as neuronal models⁴ an antagonism between PGE₁ and ACh has been detected. This suggests that such an antagonism may also have an important function in the nervous system. Third, the hybrid cells may be useful model systems for studying the biochemistry of the interaction of these and other⁷ hormones. Fourth, increases in cellular levels of cyclic nucleotides evoked by hormones are generally much higher for cyclic AMP than for cyclic GMP. Thus, in cells which strongly increase their level of cyclic AMP in the presence of a certain hormone, screening for hormone receptors controlling guanylyl cyclase may be carried out more sensitively by measuring depressed levels of cyclic AMP than by assaying cyclic GMP. Of course, a

negative result will not exclude the possibility that the hormone used stimulates formation of cyclic GMP.

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Unique DNA-binding protein in the serum of patients with various neoplasms

ONE of the major deficiencies in cancer medicine is the inability to detect low level malignant growth such as tumours at an early stage of development or residual malignant growth after therapy. Biochemical assays for malignancy have centred around carcinoembryonic antigen (CEA) and α -foetoprotein (AFP)¹, both of which are proteins found normally in embryonic tissues and only at low levels in mature, normal adults. Adult human serum contains a set of proteins with affinity for DNA² and, as we report here, foetal human serum contains additional DNA-binding proteins not observed in adult sera. Patients with a wide variety of malignant disorders have been assayed for their complement of serum DNA-binding proteins; foetal species were present in the majority of sera examined.

Human serum DNA-binding proteins were isolated by a scaled down version of our original procedure². One millilitre of serum was passed over a QAE-Sephadex column to remove immunoglobulins and the eluate, after dialysis, was applied to a column of DNA-cellulose. Proteins binding to the DNA were eluted with 0.4 M NaCl. This fraction was dialysed, reduced with mercaptoethanol in sodium dodecyl sulphate (SDS), and analysed by SDS-polyacrylamide slab gel electrophoresis³. Figure 1 shows the Coomassie blue stained slab gel of DNA-binding proteins from six normal individuals. The bands

numbered 1 and 2 are the predominant species in serum and have molecular weights of 126,000 and 94,000, respectively. The fraction at this stage is still contaminated with some albumin and immunoglobulin G. The pattern of both the major and minor protein species are invariant from individual to individual.

Foetal (cord blood) serum samples analysed in the same manner show some alterations from the pattern found in adults (Fig. 2). The most obvious change is the presence of a protein band (No. 3 in Fig. 2) of molecular weight about 36,000 with no counterpart in the adult samples. Several other changes are apparent in the minor species; a new band just below band No. 3, alterations in the pattern of bands just above albumin and between bands 1 and 2, and relatively little high molecular weight species compared with adult samples. This pattern may only approximate the actual foetal pattern since this serum was obtained from term placentae. We are proceeding under the assumption that the new species in cord blood are residual proteins from foetal development and these species may be in higher concentration in actual foetal blood.

Examination of the DNA-binding proteins in sera from cancer patients showed that foetal species were present. The most prevalent new protein in these patients was the species of foetal serum with a molecular weight of 36,000. Figure 3 shows the SDS-slab gel patterns from five individuals with various malignancies. All of these individuals exhibit a definite 36,000 species as well as other modifications in the basic adult pattern. To confirm this finding, we examined a larger number of cancer patients and scored for the presence or absence of the 36,000 species (Table 1). In all types of cancer represented, the incidence of the protein in serum is 64%. It also seems that the presence of the protein does not correlate to any particular type of malignancy, although lymphomas seem to be higher than others with a significant sample size examined. The serum samples used in this study were drawn at random from patients with and without any treatment for their malignancies. To determine if our statistics were affected by treatment, we examined the case records of 20 individuals with various carcinomas. Eleven of

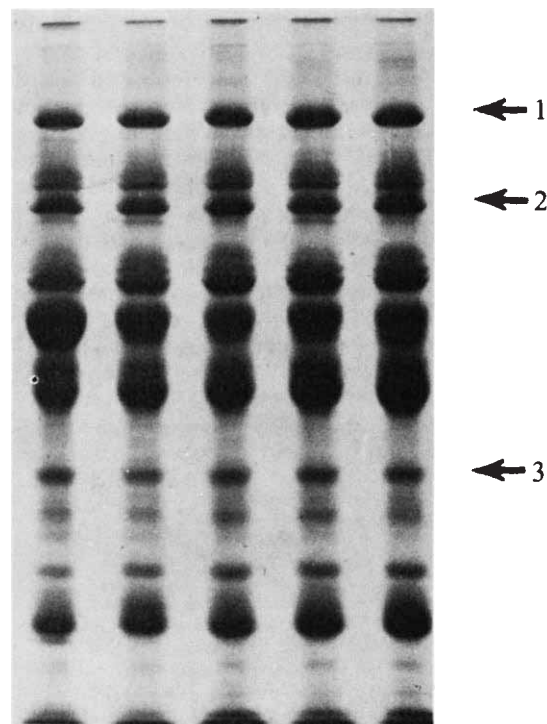


Fig. 2 SDS slab gel electrophoresis of serum DNA-binding proteins from cord blood. All samples are from individual placentae. Indications as in Fig. 1.

Fig. 1 SDS slab gel electrophoresis of serum DNA-binding proteins from normal individuals. The samples each contained 59 µg of protein. The indicated bands are: A, Albumin; H, immunoglobulin G heavy chain; L, immunoglobulin G light chain.

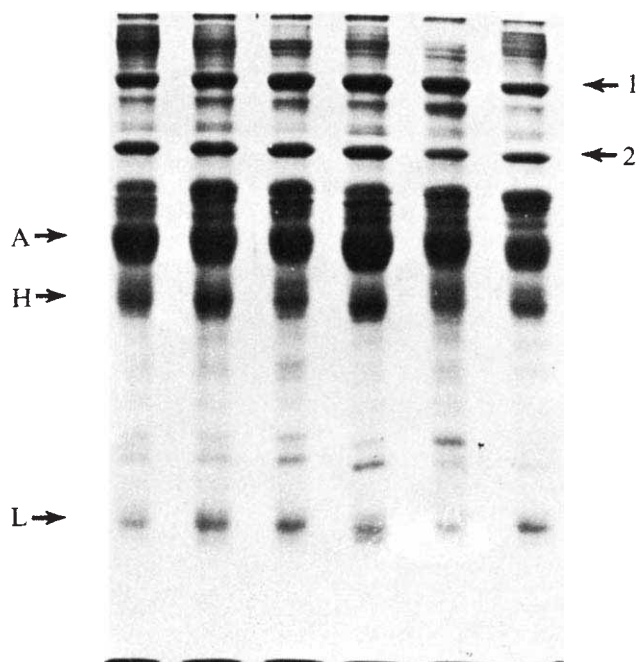


Table 1 Appearance of 36,000 molecular weight species in diagnosed malignancies

Serum source		No. positive No. tested	Overall	% Positive
Normal		0/28	0/28	0
Carcinoma Primary site:	Breast	4/11	19/31	61
	Ovary	1/2		
	Lung	5/5		
	Prostate	0/1		
	Parotid	1/1		
	Colon	2/3		
	Bone	0/1		
	Tongue	1/1		
	Pancreas	3/3		
	Multiple site	2/3		
Leukaemia Type	AML	1/2	6/11	55
	ALL	1/2		
	AGL	2/3		
	CML	1/1		
	CLL	1/3		
Sarcoma Type	Rhabdosarcoma	1/1	1/1	100
Lymphoma Type	Diffuse lymphocytic	5/7	13/17	77
	Diffuse histiocytic	3/3		
	Lymphocytic and histiocytic	1/1		
	Immunoblastic	1/1		
	Poorly differentiated	2/2		
	Macroglobulinaemia	1/3		
Hodgkin's disease		3/5	3/5	60
Multiple myeloma		1/1	1/1	100
Malignant carcinoid Somatic cell type		1/1	1/1	100

the patients were positive for the 36,000 protein and nine were negative. Among the nine negative individuals at the time the serum was drawn, six were undergoing chemotherapy and in remission, two were undergoing chemotherapy but control was uncertain, and one had no treatment. Among the eleven positive individuals, three had no treatment, six were receiving chemotherapy but were not under control, and two had a history of cancer and successful therapy. These latter two patients are of particular interest since at the time the serum sample was taken no clinical manifestations of their malignancy were evident, yet the 36,000 protein was present in their serum. Both patients, one with a breast cancer and the other with a parotid carcinoma, had obvious metastasis 6 months later.

Studies on the presence of the 36,000 protein have been qualitative since no specific assay exists at this time. Purification of this species and the development of a radioimmunoassay will enable quantitative values and a larger sample size. With the results obtained so far, it seems that the range of malignancy

In this respect, the protein appears as a generalised phenomenon regardless of the cell type or origin of the neoplastic disorder.

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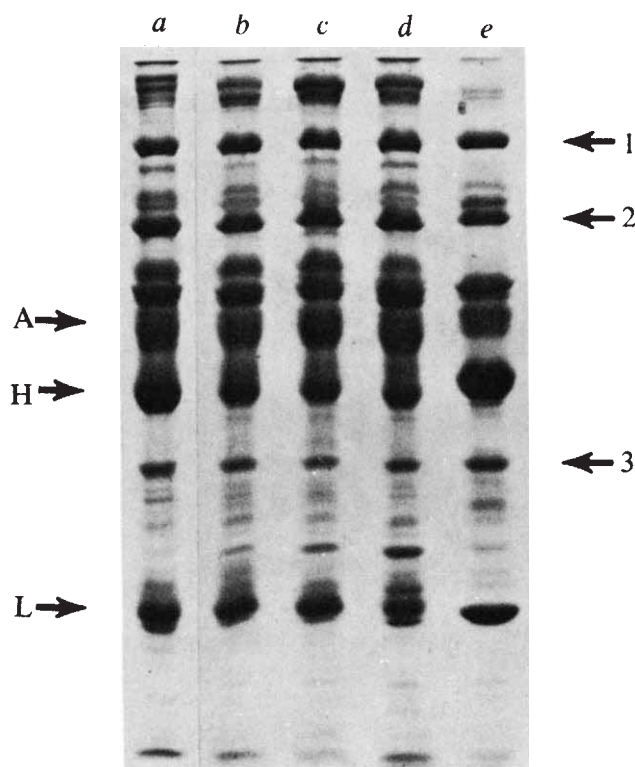


Fig. 3 SDS slab gel electrophoresis of serum DNA-binding proteins from adult individuals with various malignancies. The samples are from the sera of individuals with the following diagnoses: *a*, acute granulocytic leukaemia; *b*, histiocytic lymphoma; *c*, carcinoma of the parotid; *d*, alveolar cell carcinoma of the lung; *e*, multiple myeloma.

type in which this protein appears is broader than that observed for CEA^{4,5} or AFP⁶. In this respect, the presence of the protein may be of most use as a prognostic indicator and of limited use in specific diagnosis. Such a protein, however, may be of value in the early diagnosis of malignant growth.

The function(s) that DNA-binding proteins perform in physiological fluids normally has not been determined. It has been observed, however, that alterations in the level of DNA-binding proteins in cerebrospinal fluid can be correlated with particular diseases⁷ of the central nervous system. The increased level of the 36,000 species in the serum of patients with neoplastic diseases may be indicative of rapid cellular proliferation.

Cross idiotypic specificity among heavy chains of macroglobulins with blood group I and i specificities

THE occurrence of cross idiotypic specificity among human red cell cold agglutinins is well known¹⁻³, and there is evidence that the antigen binding site is involved in these idiotypic determinants. Two distinct idiotypic systems have been described which correlate with the combining specificities of these antibodies. The first is found among IgM cold agglutinins with blood group I and i specificities and the second among proteins of IgM and IgA class with blood group Pr specificities². The idiotypic determinants in each system are numerous and variable numbers are expressed on proteins from different individuals²⁻⁴. Sensitive binding assays showed one idiotypic determinant to be common to at least 73% of cold agglutinins with specificity for the I-i antigen complex³. With the antisera raised against the intact proteins the relative roles of the heavy and light chains in the cross-specificity determinants could not be ascertained because both chains in combination were required for their expression². In the studies reported here, rabbit antisera against the isolated heavy chains of an anti-I and an anti-i cold agglutinin have been used and cross idiotypic specificity among the heavy chains of this group of antibodies has been demonstrated.

Cold agglutinins and macroglobulins without cold agglutinin activity were isolated and their fragments prepared as described previously². The isolated heavy and light chains were dialysed against distilled water; one half of each preparation was lyophilised and the other half rendered soluble in isotonic solution by carbamylation². Rabbit antisera were raised against the untreated and carbamylated heavy chains of an anti-I cold agglutinin Low^{2,3}, and an anti-i cold agglutinin Nic^{2,3} as described previously². The use of carbamylated H and L chains enabled quantitative precipitin, gel diffusion and haemagglutination tests to be carried out in isotonic solutions.

In gel precipitation tests all four antisera showed activity against determinants revealed on the isolated H chains which were not available on the intact proteins (Fig. 1). After absorption at equivalence with the heavy chains of two non-cold agglutinin macroglobulins and of human Cohn Fraction II, the antisera no longer precipitated with any of the isolated H chains, nor with the intact cold agglutinins. By passive haemagglutination (HA) assays, however, they showed specificity for cold agglutinin H chains (Table 1). The two absorbed anti-Nic H sera showed individual as well as cross specificity (HA titres of 1,024-2,048 with the homologous Nic H chains and 64-256 with Low H chains). The two absorbed anti-Low H sera, on the other hand, showed cross specificity only (HA titres of 128-512 with both Low and Nic H chains). The individual

Table 1 Haemagglutination titres of the absorbed antisera to the H chains of cold agglutinins Low and Nic

H chain coat*	Antisera against			
	Untreated	Low H chains Carbamylated	Untreated	Nic H chains Carbamylated
Cold agglutinins				
Low	512†	128	64	256
Nic	512	128	1,024	2,048
Non-cold agglutinins				
IgM Sto	<4	<4	<4	<4
IgM Cab	<4	<4	<4	<4
Cohn Fr II	<4	<4	<4	<4

The antisera were absorbed at equivalence (as determined by quantitative precipitin² assays) with the H chains of non-cold agglutinin macroglobulins Sto² and Cab³ and of human Cohn Fraction II.

*Tanned human red cells⁵ were coated with carbamylated H chains.

†Titres (reciprocals of dilutions) determined by the microtitre⁶ method.

Table 2 Haemagglutination inhibition assays with absorbed antisera to H chains of cold agglutinins Nic and Low

H chain inhibitors	Antisera against					
	Nic H untreated		Nic H carbamylated		Low H untreated	Low H carbamylated
	Nic H coat (1:64)	Low H coat (1:4)	Nic H coat (1:128)	Low H coat (1:8)	Low H coat (1:32)	Low H coat (1:8)
	minimum concentration giving complete inhibition ($\mu\text{g ml}^{-1}$)					
Cold agglutinins						
Nic	400	25	400	25	6	50
Low	>1,015	127	>1,015	254	32	127
Kroo	>1,035	129	>1,035	65	32	32
Non-cold agglutinins						
IgM Sto	>965	>965	>965	>965	>965	>965
IgM Cab	>836	>836	>836	>836	>836	>836
Cohn Fr II	>1,085	>1,085	>1,085	>1,085	>1,085	>1,085

The figures in parentheses indicate the antiserum dilutions used with the various H chain coats.

idiotypic specificity of the two anti-Nic H sera was confirmed in inhibition of HA assays where the homologous H chain (Nic) was used as the red cell coat (Table 2): the H chains of Nic were inhibitory while those of cold agglutinins Low and

Kroo³ were not inhibitory at the concentrations tested. On the other hand, when a heterologous cold agglutinin H chain (Low) was used as the protein coat, the two anti-Nic sera clearly showed cross idiotypic specificity among the H chains of cold agglutinins Nic, Low and Kroo. The anti-Low H sera again showed cross idiotypic specificity only: all three cold agglutinin H chains could inhibit the haemagglutination of the homologous H chain coat. The specificities of the four antisera, whether raised against the untreated or the carbamylated H chains, were satisfactorily demonstrated using carbamylated H chains as antigens.

Further studies were carried out to confirm the specificity of the absorbed antisera for dissociated cold agglutinin H chains (Table 3). Isolated L chains of the three cold agglutinins were not inhibitory at the concentrations tested; nor were whole cold agglutinin molecules or fragments with intact HL chain bonds; however, after reduction of whole cold agglutinins and chain dissociation by the method of McLaughlin and Solomon⁷, inhibitory activity was revealed. The HA inhibition studies were therefore extended to include five additional anti-I cold agglutinins and four non-cold agglutinin macroglobulins after reduction, alkylation and chain dissociation. With the exception of anti-I Ma the cold agglutinins tested by this method showed inhibitory activity and could be clearly distinguished from the non-cold agglutinin proteins (Table 3). These findings are in agreement with previous studies^{2,3} using idiotypic antisera against intact proteins which showed that these cold agglutinins, with the exception of Ma, had strong idiotypic cross reactions; anti-I Ma which differs markedly from the other cold agglutinins in its antigen binding specificity^{8,9} was shown to be deficient in some of the idiotypic determinants associated with these proteins.

The idiotypic determinants recognised by the anti-H chain antisera seem to be different from those recognised by antisera

Fig. 1 Gel precipitation reactions of an unabsorbed antiserum (A) raised against the untreated H chains of cold agglutinin Nic. The peripheral wells contained native cold agglutinin Nic (1 mg ml^{-1}), carbamylated H chains of cold agglutinins Nic, Low, Kroo³ and of the non-cold agglutinin macroglobulin Cab³ (0.2 mg ml^{-1}). The Nic H precipitin line spurs over that formed by intact IgM Nic indicating that the antiserum has activity toward additional determinants present on the isolated H chains. Similar reactions were obtained with the other three antisera raised against the untreated or the carbamylated cold agglutinin H chains.

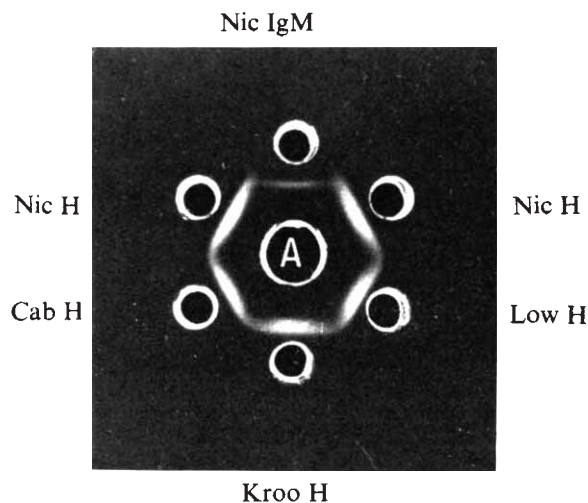


Table 3 Haemagglutination inhibition assays with an absorbed anti-serum to untreated H chains of cold agglutinin Low showing cross idiotypic specificity for reduced-dissociated H chains (red cell coat-Low H chains; antiserum dilution 1:32)

Inhibitors	Minimum concentration giving complete inhibition ($\mu\text{g ml}^{-1}$)
Cold agglutinins	
Low H chains	20
Low L chains	> 1,000
Low Fab	> 1,230
Low F(ab') ₂	> 1,238
Low IgM reduced-dissociated*	18
Nic† H chains	24
Nic L chains	> 1,000
Nic IgM	> 1,000
Nic Fab	> 1,280
Nic F(ab') ₂	> 2,840
Nic IgM reduced-dissociated	60
Step IgM reduced-dissociated	17
Da IgM reduced-dissociated	950
Sch‡ IgM reduced-dissociated	500
Den†‡ IgM reduced-dissociated	140
Ma IgM reduced-dissociated	> 1,750
Non-cold agglutinins	
Mi IgM reduced-dissociated	> 7,800
Gr IgM reduced-dissociated	> 5,000
Dav‡ IgM reduced-dissociated	> 8,850
Ham‡ IgM reduced-dissociated	> 8,000
Cohn Fr II reduced-dissociated	> 5,000

*HL dissociation without chain separation was carried out by reduction, alkylation, dialysis against 1 M acetic acid followed by dialysis against distilled water, as previously described⁷.

†Anti-i cold agglutinin; all others had anti-I specificity.

‡IgM λ proteins; all others were IgM κ .

raised against the intact cold agglutinins. The latter fail to react with dissociated H and L chains². The findings with the anti-H chain sera strongly suggest that the H chains of this group of antibodies with related antigen bindings specificities have similarities in their hypervariable regions—the absorption procedures and the negative findings with the H chains of pooled human IgG would make V_H subgroup specificities¹⁰ in the absorbed antisera unlikely.

Similar studies now under way with the light chains of these antibodies may provide additional important information regarding the selection of hypervariable regions in these antibodies, some of which have selected κ chain subgroups and others λ chains^{2,3,12}.

In highly inbred strains of mice the H and L chains of myeloma proteins with the same antibody activity and the same idiotype determinants usually have identical or very similar V regions, that is, identical V_L and V_H subgroups (framework regions) as well as identical or very similar hypervariable regions^{13,21} (site determining regions). In partially inbred rabbits, however, occasional proteins with differing V region subgroups or allotypes have been reported which show cross-idiotype specificity¹⁴⁻¹⁶; these have provided support for the insertional model¹⁷ for the coding of the site determining regions of antibodies. This model proposes that separate genes code for the site-determining and the framework portions of the V region. The cold agglutinins and certain other antibodies with restricted specificities¹⁸⁻²⁰ in unrelated persons offer an excellent opportunity for studying the selection of site determining regions in relation to other V region structures on H and L chains. The availability of discriminating idiotype antisera against isolated chains should prove useful in the screening and selection of H and L chains and their fragments for variable region sequencing.

ROBERT CHILDS
TEN FEIZI

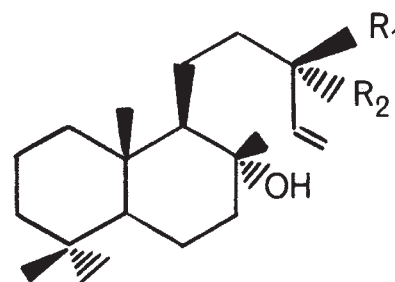
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Errata

In the article "Control of rust diseases by diterpenes from *Nicotiana glutinosa*" by J. A. Bailey, G. A. Carter, R. S. Burden and R. L. Wain (*Nature*, **255**, 328; 1975) Fig. 1 was incorrect. The correct form is reprinted below.



In the article "Observations of a nonlinear interaction involving three electromagnetic waves in a laboratory magnetoplasma" by P. J. Christiansen, M. J. Giles, G. Martelli and N. D. Wells (*Nature*, **254**, 685; 1975) there was an error in Fig 3. The correct figure and legend are reprinted below.

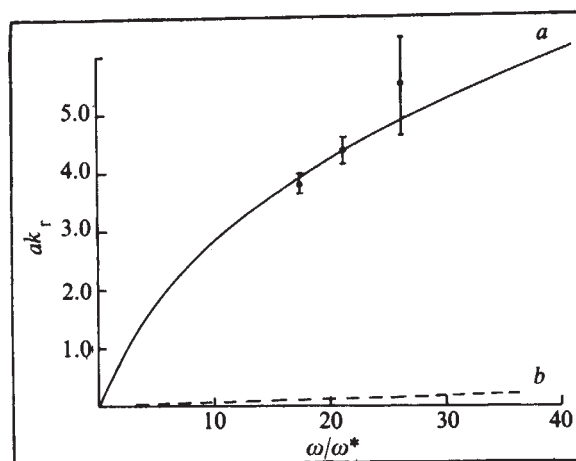


Fig. 3 Experimental data fitted to the theoretical dispersion curve (a) of low frequency whistlers (helicon waves⁶). In the axes labels a is the tube radius, k_r is the real part of the wavenumber and $\omega^* = B_0/4\pi nea^2$. The inferred value of the plasma frequency, n ($\sim 1.2 \times 10^{12} \text{ cm}^{-3}$) is in good agreement with independent Langmuir probe measurements¹. b. Dispersion curve for quasistatic waves.

reviews

HERE is a book that should immediately be put into paperback and placed in the hands of undergraduates everywhere and in all disciplines. Its subject matter ranges from the cathedral builders of the Middle Ages to the prophets of systems analysis in our own century. Yet Dr Pacey is nowhere superficial. His discussion of "A century of invention: 1250-1350" includes, for example, an exciting examination of the thesis that the weight-driven clock was not the product of monastic needs but was inspired by the imaginative desire to parallel the movements of the planets in a model here below. The author constantly stresses non-economic motivation in the development of technology and, in this respect, seems to share a point of view with that great historian of metallurgy, C. S. Smith, whose work he never cites. It is very refreshing, moreover, to see a history of technology that refers truly to the technology of Asia (China, India and Japan) as well as that of the West, and moves with confidence in discussing the Islamic contributions to mediaeval and renaissance technology. One chapter is entitled "The effects of mechanisation in Europe and Asia: trends in technology between 1810 and 1870".

Though Dr Pacey is best known for papers on various aspects of the Industrial Revolution in Britain, it is in his discussion of mediaeval and ren-

Weighted clocks and steam engines

Eric H. Robinson

The Maze of Ingenuity: Ideas and Idealism in the Development of Technology. By Arnold Pacey. Pp. 350. (Allen Lane: London, January 1975.) £5.50.

naissance architecture that he excels in this book. Not that he does not have illuminating comments on John Smeaton, James Watt, Peter Ewart, William Strutt and other well known figures of the late eighteenth and early nineteenth centuries; but his discussion flows most freely when he is not too closely involved with biographical detail. The book seems to be more concerned with broad imaginative ideals within different cultures and with the state of craftsmanship in different civilisations than it is with the heroic inventor, yet the biographical approach has a strong hold upon the author and sometimes seems to run away with him. On occasion, indeed, it seems to lead to self-contradiction. For example, on page 226, Dr Pacey quotes from a letter by George

Lee to illustrate "the abysmal ignorance of algebra and the calculus which prevailed among even well-read engineers in Britain at this time" but on page 240 quotes with approval Robert Owen's judgment of Lee as "one of the most scientific men of his day, who was considered a man of very superior attainments, having been highly educated, and being a finished mathematician".

I would question in particular Dr Pacey's evaluation of some eminent technologists, but found the book constantly illuminating, soundly researched, ranging widely over different disciplines and always civilised in tone. How many historians of technology could be expected to comment with insight upon the poetry of Alexander Pope? Dr Pacey, however, is wrong in drawing a contrast between Britain's economically-motivated uses of the steam engine for mills and the Russian or French use of it to water gardens. One of the first uses of the Boulton and Watt steam engine in Britain was to water a landed gentleman's garden, and if you are pumping water you may as well use it to refresh your carnations or your cabbages as to drive a loom or power a brewery. In fact some of Dr Pacey's differentiations between technology motivated by ideals and technology motivated by gain are a little too simplistic but this is the best book on the history of technology over a wide period to appear for a long time.

THIS book results from an Interdisciplinary conference on amniotic fluid held in May 1973, under the auspices of the American Association of Clinical Chemists, and the Chicago Gynaecological, Immunological and Paediatric Societies. Each session is represented by a section of the book.

Section 1 includes papers on the morphology and development of the amnion, and the dynamics and chemistry of the amniotic fluid. The latter is concerned mainly with a consideration of the commoner metabolites although there is a report of recent work on measurements of adrenalin and noradrenalin.

Section 2 includes papers on immunoglobulins and other protein constituents of amniotic fluid, electrophoretic and immunological analysis of amniotic fluid, placental lactogen, disseminated intravascular coagulation in obstetrics, and papers dealing with problems arising from Rhesus incom-

Amniotic fluid

R. W. E. Watts

Amniotic Fluid: Physiology, Biochemistry and Clinical Chemistry. (Current Topics in Clinical Chemistry, vol. 1.) Edited by Samuel Natelson, Antonio Scommegna and Morton B. Epstein. Pp. xv+386. (Wiley: New York and London, 1974.) £10.80.

patibility. Section 3 deals with the obstetrical problems such as the technique of amniocentesis, use of ultrasonography, the value of lecithin, sphingomyelin and oestrogen measurements in clinical practice.

The last section deals with the very rapidly growing field of the diagnosis of genetic disorders, and there have been new developments even in the small range of topics covered since the conference was held. Newton

Ressler's contribution discusses a pitfall which must be avoided in this work and it should be read carefully by anyone entering the field. Abraham Saifer and his colleagues describe their extensive experience of mass screening for heterozygous carriers of Tay Sachs disease, and in the prenatal diagnosis of this disorder. This chapter is of particular interest as a pointer to what can be achieved when necessary in this area of preventive medicine.

The book contains much of interest to biochemists and clinicians, but it is patchy and uneven in coverage and in style. Some of the contributions would have profited from more severe editing so that they less obviously resembled a transcript of the spoken contribution. Regrettably, a few of the papers contain mainly material which is already well known. It is, however, a useful book for the specialist and for the library. □

Structure and role of plasma proteins

Structure and Function of Plasma Proteins, vol. 1. Edited by A. C. Allison. Pp. viii+316. (Plenum: London and New York, 1974.) \$28.90.

As the title indicates, this book is concerned with both the structure and the function of plasma proteins. Unfortunately, with the exception of the immunoglobulins and albumin, available structural data are still mainly of a physiochemical character but even so information of this kind is well presented for a number of plasma proteins. Most, though not all, selected for inclusion have at least one known function. It is evident that in several cases these functions and their normal physiological roles are identical, though in other cases true physiological roles are still uncertain; the existence of analbuminaemics who often enjoy excellent health must serve to underline this point. In this regard it is disappointing that the chapter on albumin deals only with "aspects of chemical behaviour and structure" and that, in consequence, no comment is made on the function of albumin. It is indeed curious that a book with such a title does not as yet include any information concerning the function of that protein which is present at the highest concentration in plasma. But at least the information that is given about albumin is excellently presented and includes an interesting discussion of the macro and microheterogeneity of this protein. The opposing views of two schools of thought on the complex matter of the pH dependent N-F transition of albumin are very fairly presented.

Very satisfactory descriptions are given of present knowledge concerning both haptoglobin and haemopexin, two proteins usually considered to be of known function. As with albumin, genetic deficiency (or at least very low levels of haptoglobin) is compatible with reasonable health and it certainly is "important to know if complete absence of haptoglobin is compatible with life".

An especially warm welcome must be given to the chapter on acute phase reactants. As I have mentioned, the physiological roles of certain of these proteins, such as fibrinogen, are clear whereas the stimuli which bring about increased synthesis after injury of this and other acute phase proteins are little understood. Although on the basis of experiments using the isolated perfused rat liver it has been suggested that hormonal factors are of prime importance, evidence obtained by perfusion of physiologically normal livers suggests otherwise; a very rapid rate of

synthesis of haemopexin has been obtained when such livers are perfused (p. 42). Since haemopexin is not an acute phase reactant the finding that in the presence of hormones perfused livers from normal rats synthesise acute phase reactants at very high rates falls into a new perspective. Evidently, the simplest hypothesis at this time is that perfused livers synthesise many proteins (albumin is a major exception) at a greatly increased rate. Such behaviour may most reasonably be ascribed to the presence *in vivo* of inhibitors which cease to function under perfusion conditions.

In spite of the fact that certain chapters are out of date the book represents a valuable contribution to the field.

A. H. Gordon

Life and science in cold environments



Glaciated arctic landscape on the north-eastern coast of Baffin Island.

Arctic and Alpine Environments. Edited by Jack D. Ives and Roger G. Barry. Pp. xviii+999+47 plates. (Methuen: London, November 1974.) £35.00.

In recent years Man and his technology have begun to invade the cold arctic regions for science, minerals and simply for amenity. So this book, dedicated to Professor Carl Troll in acknowledgment of his extensive work on altitude geology, is a very timely synthesis of arctic and alpine work. It is edited by the director and coworker of the Institute of Arctic and Alpine Research at the University of Colorado and is largely written by colleagues working there or at government departments or universities in the USA or Canada; two authors are German, one Norwegian and one a New Zealander. Each contributor is an authority in his field and is very readable and generally succinct; some of the chapters are scholarly masterpieces of condensation.

The awful price of £35.00 will deter

most private purchasers but it is only the absence of exploration sagas and of contributions concerning social sciences and economic aspects that prevents the book from qualifying as an encyclopaedia. The 1,000 pages, which include a glossary, an index and 49 black and white plates inconveniently located at the end, embrace 37 papers, each of which has an extensive bibliography. They are divided into sections covering present and past environments, present biota and its development, abiotic processes, man in cold environments and man's impact on the environment. A more practicable approach would have been to present a format in three parts covering physical, biological and human (including technological) aspects. Expensive photographs could have been replaced with sketches where possible, and the number of diagrams could have been increased, even though 200 are already included.

Some of the papers treat elements of the environment in a systematic manner; the paper entitled "Snow" is an erudite summary of this ubiquitous characteristic of both arctic and alpine environment, but it says nothing of drift snow and would benefit from more diagrams. On the other hand regional themes dominate other papers, such as those on "Arctic Hydrology" and "The Present Ice Cover". Many of the papers, however, combine both the systematic and the regional approach—that on "Radioecology", for example—but very few treat 'arctic' and 'alpine' areas as similar regions; indeed, one of the achievements of the book is to pinpoint the differences between them. One of the longest and most fascinating papers explains how the alpine and arctic tundra are combined in a single tundra biome, an approach which, though having some justification where plants are concerned, has far less in the case of animals, particularly vertebrates, as not a single mammal is common to both arctic and alpine tundra. Controlling the differences between the two environments are length of day, solar radiation patterns, topography and moisture. Distances involved are so much greater in the arctic and it encloses an ocean which (even though largely ice covered) provides a further contrast.

Extremely brief conclusions from many atmospheric models and palaeotemperature profiles form only a small part of a brilliant precis on palaeoclimatology. Radiation over all the different time scales is a dominant variable in the many heat balance expressions used through the book. Better coordination of these would be a blessing to the student reader. And more data from The International Biological Programme, from the Alaska pipeline and from many smaller projects is now available to the rapidly expanding, corporate body of knowledge so lucidly defined in this most creditable collection.

H. Lister

Introduction to Biophysical Plant Physiology. By P. S. Nobel. Pp. xii+488 (W. H. Freeman: San Francisco and Reading, 1974.) £7.10.

THIS excellent book has been written essentially for advanced undergraduate and postgraduate study and is an extension of an earlier publication by the same author (*Plant Cell Physiology: A Physiochemical Approach*: Freeman, 1970). Its aim is to show how basic physical and chemical principles can be applied to whole plant and plant cell physiology.

The first three chapters are extended and improved versions of the corresponding chapters in the original book and give thorough descriptions of the quantitative and experimental aspects of water and ion distribution and move-

ments in plant cells. These phenomena are discussed in terms of both classical and irreversible thermodynamics. As in the earlier edition, Chapter four gives an outline of the properties of light and its absorption by molecules and serves as an introduction to chapters five and six. These deal with the light reactions of photosynthesis and the main features of energy conversion in the chloroplast and mitochondrion.

The remaining chapters are additions to the original book and cover various aspects of leaf and whole plant interaction with the environment, concentrating particularly on water, gase-

ous and energy fluxes within and at the surfaces of the plant.

The book contains a number of numerical examples for the reader to test his understanding of the concepts presented.

The text itself is fairly readable, although to some extent there is inconsistency in the degree of explanation of some of the concepts presented. Overall, I would like to congratulate the author for producing a unique and valuable text-book. It is reasonably priced and I thoroughly recommend it to all those interested in a quantitative approach to plant physiology—both at the teaching and research level—who wish to place this aspect of plant biology on a firmer physical and chemical basis.

J. Barber

The Vanishing Lichens: Their History, Biology and Importance. By D. H. S. Richardson. Pp. 231. (David and Charles: Newton Abbot, London and Vancouver, 1975.) £5.25.

THE title of this book is unfortunately very apt. No other major group of plants has suffered so much from the effects of atmospheric pollution. The area of distribution of many species has contracted dramatically during the past century and all but the few pollution-tolerant species really are vanishing—and not all that slowly.

But this is not a book about the effects of pollution on lichens. Rather, it gives a readable and enjoyable account of the group as a whole; because it is deliberately aimed at a popular audience, there is a necessarily heavy emphasis upon the importance of lichens to man and how he uses them.

The book is assuredly successful in achieving its objective. Furthermore, there is much even to intrigue specialists such as myself. Would that more scientists took time off to write books which are genuinely entertaining yet keep within the bounds of scientific accuracy. In the long run it will be books like this which will help the cause of conservation amongst the public at large, rather than patronising diatribes in the Sunday colour supplements. People need to develop affection for wild life, not just a dutiful respect.

There is a slight air of zaniness—characteristic of quite a number of other books published by David and Charles; one can't help feeling that James Thurber selected some of the line drawings. It is a pity, though, that the need to save money has forced the publishers to use poor or just bad reproduction of plates which were very good in the original. The picture of lichens colonising power-station cooling-towers on page 7 means little.



Lichenologists at work; from Richardson's book.

Curiously, the farther the author gets away from explaining science, the better he is. Particularly fascinating are the many little side-trips away from lichens, that occur in the narrative. Thus, a description of the use of lichens for dating Easter Island megaliths goes on, out of sheer interest, to say something about megaliths in their own right.

Dr Richardson is to be congratulated on writing the most entertaining book on lichens to date.

D. C. Smith

Crop Physiology: Some Case Histories. Edited by L. T. Evans. (Cambridge University Press: London, February 1975.) £8.00; \$23.50.

IN a world in which a depression in the crop yield of one major food-producing country can have international repercussions or even result in mass starvation, books which critically evaluate important aspects of current knowledge of agricultural crops are to be welcomed.

In this book, authoritative contributors from Australia, the USA and Japan examine the physiological basis of yield in nine important crops: maize, sugar cane, rice, wheat, soybeans, pea, potato, sugar beet and cot-

ton. The introductory chapter deals with some more general aspects of world food supply, crop evolution and the origins of crop physiology. In the final chapter Dr Evans discusses and collates the information obtained about individual crops and considers possible applications to agronomic techniques and plant breeding. Each chapter is followed by an extensive list of references.

The crops chosen illustrate the diversity of 'yield' in the agricultural sense: the tubers of the potato, the sucrose content of the sugar cane stem, grains of the cereals, and fibres of the cotton boll are all considered. The physiological pathways and limitations to attaining maximum yields are equally diverse and the separate chapters discuss these topics from rather contrasting viewpoints, ranging from the largely agronomic (maize) to the more theoretical approach of crop modelling (sugar beet). Most chapters are not confined to purely physiological aspects and include useful sections on origins, evolution, adaptability and distribution of the crop and on many aspects of growth from sowing to harvest. There is a strong awareness throughout the book of the potential applications to plant breeding arising from current knowledge of crop physiology. This is emphasised by Dr Evans who stresses the value of the production of new ideotypes, discusses the bases of yield assessment—he considers that the efficiencies of water use and of phosphate use will become more important in the future—and briefly examines the efficiency of energy use in agriculture.

The book is extremely readable and well presented and can be recommended not only to crop physiologists but also to agronomists and plant breeders.

David Wilson

announcements

International meetings

August 3-9, **Nutrition**, Kyoto, Japan (Professor H. Mitsuda, Research Institute for Production Development, Morimoto, Shimagamo, Sakyoku, Kyoto 606, Japan or Kyoto International Conference Hall, Takara-ike, Sakyoku, Kyoto 606, Japan).

August 4-7, **Neutron activation analysis**, Cambridge (Mr C. H. Gill, Conference Secretary, Neutron Division, Marconi Elliott Avionic Systems Ltd, Elstree Way, Borehamwood, Herts WD6 7RX, UK or Dr J. Pauwels, Euristop Office, Commission of the European Communities, 200 rue de la Loi, B1040 Brussels, Belgium).

August 4-7, **Image processing for 2D and 3D reconstruction from projections**, California (Optical Society of America, 2000L Street, NW, Washington, DC 20036).

August 4-7, **Odonatology**, Lancaster (Dr T. T. Macan, Freshwater Biological Association, Ferry House, Far Sawrey, Ambleside, Cumbria, UK).

August 4-9, **Biophysics**, Copenhagen (Fifth International Biophysics Congress, c/o DIS Congress Service, 3 Knabrostaede, DK 1210 Copenhagen K, Denmark).

August 5-6, **Neutron diffraction**, Petten, Netherlands (Neutron Diffraction Conference, Reactor Centrum Nederland, Petten (NH), The Netherlands).

August 6-8, **Applications of X-ray analysis**, Denver, Colorado (Dr C. O. Ruud, Metallurgy and Materials Science Division, Denver Research Institute, University of Denver, Denver, Colorado 80210).

August 7-14, **Crystallography**, Amsterdam (J. N. King, Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, UK or Organisatie Bureau Amsterdam Bv, PO Box 7205, Amsterdam, The Netherlands).

August 11-15, **Astronomy**, Leicester, UK (Professor K. A. Pounds, Chairman, Local Organising Committee, Department of Physics, University Road, Leicester LE1 7RH, UK).

Person to Person

Somantin. Researcher concerned about possible implications of theories concerning somantin and cataglykin (fragments of growth hormone which may be important in control of blood glucose concentration, and development of some cases of diabetes), wishes to know results of relevant studies, besides those of Bornstein: *Nature*, **217**, 707 (1968) or Bornstein, J., *et al.*, *Postgrad. med. J.*, **49** (suppl.), 219-242 (1973), (Dr P. L. Schwartz, Department of Clinical Biochemistry, University of Otago Medical School, PO Box 913, Dunedin, New Zealand).

Biochemical journal. Volumes 62-105 (1956-67), complete, unbound, will be presented (delivered) to research laboratory or educational institution 'with a good cause'. Applicants should be prepared to bind volumes and indicate source of presentation. Apply by June 30 to: Dr R. Bernstein, SA Institute for Medical Research, PO Box 1038, Johannesburg, South Africa.

Leeds house. Academic going on sabbatical seeks family for house in Leeds. From August, 1975-September, 1976. Possible to commute to Bradford or York. Four bedrooms, large kitchen and reception rooms, cellars (for hobbies or storage), large garden, garage (Dr S. Baumberg, Department of Genetics, University of Leeds, Leeds LS2 9JT, Yorkshire, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robbie Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

University of Oxford. Eleventh Annual Report of the Delegates of the Science Area for the year ending 31 July 1974. (Supplement No. 1, to the *University Gazette*, Vol. cv, March 1975.) Pp. 139. (Oxford: The University, 1975.) £1.25. [14]

Greenwich Observatory: 300 Years of Astronomy. Edited by Colin A. Ronan. (An Exhibition at the National Maritime Museum telling the story of Britain's oldest scientific institution, the Royal Observatory, at Greenwich and Herstmonceux.) Pp. 63+16 coloured plates. (London: Times Books, 1975.) [24]

The effect of Man on the Landscape: the Highland Zone. Edited by J. G. Evans, Susan Limbrey and Henry Cleere. (Research Report No. 11.) Pp. vii+129. (London: The Council for British Archaeology, 1975.) £7.50. [34]

University of Oxford. Annual Reports, 1972-4. Pp. 20. (Oxford: The University, 1975.) £1. [44]

Science Research Council. List of Research Grants Current on 1 October 1974. Pp. 182. (London: Science Research Council, 1975.) [74]

Daresbury 1974—Progress Report. Pp. 52. (Daresbury, Warrington: Science Research Council, Daresbury Laboratory, 1975.) [74]

The Zoological Society of London. Annual Report 1974. Pp. 48. (London: The Zoological Society of London, 1975.) [104]

Flora of Tropical East Africa. Edited by R. M. Polhill. Cannabaceae. By B. Verdcourt. Pp. 3. 15p. Cochlospermaceae. By B. Verdcourt. Pp. 3. 15p. (London: Crown Agents for Oversea Governments and Administrations, 1975.) [104]

The United States Binary Nerve-Gas Programme: National and International Implications. By Julian Perry Robinson. (ISIO Monographs, First Series, No. 10.) Pp. 51. (Brighton: Institute for the Study of International Organization, University of Sussex, 1975.) £1.50; \$4. [104]

Maincrop Potato Production, 1973. Pp. 47. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) 25p. [104]

The Macaulay Institute for Soil Research. 1973/1974 Annual Report. (No. 44). Pp. 95. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1975.) [104]

Natural Environment Research Council. National Institute of Oceanography, Collected Reprints, Vol. 21. Reprints 886-952. (Wormley, Godalming: National Institute of Oceanography, 1973.) [104]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1280: An Application of Normal Mode Theory to the Retrieval of Structural Parameters and Source Mechanisms from Seismic Spectra. By F. Gilbert and A. M. Dziewonski. Pp. 187-269. UK £3.50; Overseas £3.60. Vol. 278, No. 1281: Field Emission and Field Ionization in Liquid ⁴He. By A. Phillips and P. V. E. McClintock. Pp. 271-310. UK £1.70; Overseas £1.75. (London: The Royal Society, 1975.) [114]

Fundamental Research in an Industrial World. By Professor Alastair Cameron. (Inaugural Lecture, 22 January 1974.) Pp. 199-210. (London: Imperial College of Science and Technology, University of London, 1975.) 60p. [114]

Other Countries

Annual Report of 1972, 1973, of the Keikyū Aburatsubo Marine Park Aquarium, No. 5, 6. Pp. 47. (Miura City, Kanagawa, Japan: The Keikyū Aburatsubo Marine Park Aquarium, 1975.) [44]

New Theory of Faster-than-Light Relativity (Classical and Quantum) with Wonders of Implied Results for Public Interest and Understanding. By Dr. B. B. P. Sinha. Pp. 92. (Guelph, Ontario: Institute of Science and Mathematics, PO Box 1574, 1974.) [44]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 237: Carboniferous Ammonoites and Stratigraphy in the Canadian Arctic Archipelago. By W. W. Nassichuk. Pp. 240 (18 plates). \$7. Memoir 376: Geology of the Antigonish Highlands, Nova Scotia. By D. G. Benson. Pp. 92. \$4. Memoir 377: Geology of Kognak River Area, District of Keewatin, Northwest Territories. By K. E. Eade. Pp. 66 (13 plates). \$4. (Ottawa: Information Canada, 1974 and 1975.) [44]

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